JOURNAL OF DAIRY SCIENCE

Contents

Studies on Oxidized Milk Fat. I. Observations on the Chemical Properties of the Volatile Flavor Material from Oxidized Milk Fat. MARK KEENEY AND F. J. DOAN 713

Studies on Oxidized Milk Fat. II. Preparation of 2,4-Dinitrophenylhydrazones from the Volatile Material from Oxidized Milk Fat. MARK KEENEY AND F. J. DOAN 719

Studies on Oxidized Milk Fat. III. Chemical and Organoleptic Properties of Volatile Material Obtained by Fractionation with Various Solvents and Girard's Reagent. MARK KEENEY AND F. J. DOAN 728

The Effect of Vitamin D on the Utilization of Energy and Protein of the Ration of Calves. N. F. COLOVOS, H. A. KEENER, A. E. TEERI AND H. A. DAVIS 735

Determination of Heat-produced Reducing Substances in Milk with Porphyrexide. AUBREY P. STEWART, JR. 743

Vitamin B_{12} in Blood of Newborn and Colostrum-fed Calves and in Colostrum and Normal Milk of Holstein and Jersey Cows. W. B. ANTHONY, J. R. COUCH, I. W. RUPEL, MARY B. HENDERSON AND CHARLESTA BROWN 749

Enumeration of Lactic Streptococcus Bacteriophage by a Two-layer Plate Method for Plaques. G. E. TURNER AND F. E. NELSON 754

The Fertility of Bovine Semen in Diluters Containing Varying Amounts of Egg Yolk. JOHN O. ALMQUIST 763

Flexible Wrappers for Cheddar Cheese. DARRELL D. DEANE AND P. A. DOWNS 767

Preliminary Studies of the Effect of Acido-proteolytic Organisms and Temperatures of Curing on the Ripening of Cheddar Cheese Made from Pasteurized Milk. DARRELL D. DEANE 776

The Solar-activated Flavor of Homogenized Milk. V. The Electrophoretic Analysis of a Contributing Minor-protein Fraction. BERNARD R. WEINSTEIN, H. A. LILLEVIK, C. W. DUNCAN AND G. M. TROUT 784

Electrophoretic Studies of Commercial and Iodinated Casein. SYED KAMAL AND C. W. TURNER 790

Electrophoretic Study of Bovine Thyroglobulin and Iodinated Bovine Gamma Globulin. SYED KAMAL AND C. W. TURNER 798

Permanent Pipe Lines for Dairy Plants. DONALD R. MOORE, P. H. TRACY AND Z. JOHN ORDAL 804

The Deaeration of Raw Whole Milk before Heat Treatment as a Factor in Retarding the Development of the Tallowy Flavor in its Dried Product. GEORGE R. GREENBANK AND PHILIP A. WRIGHT 815

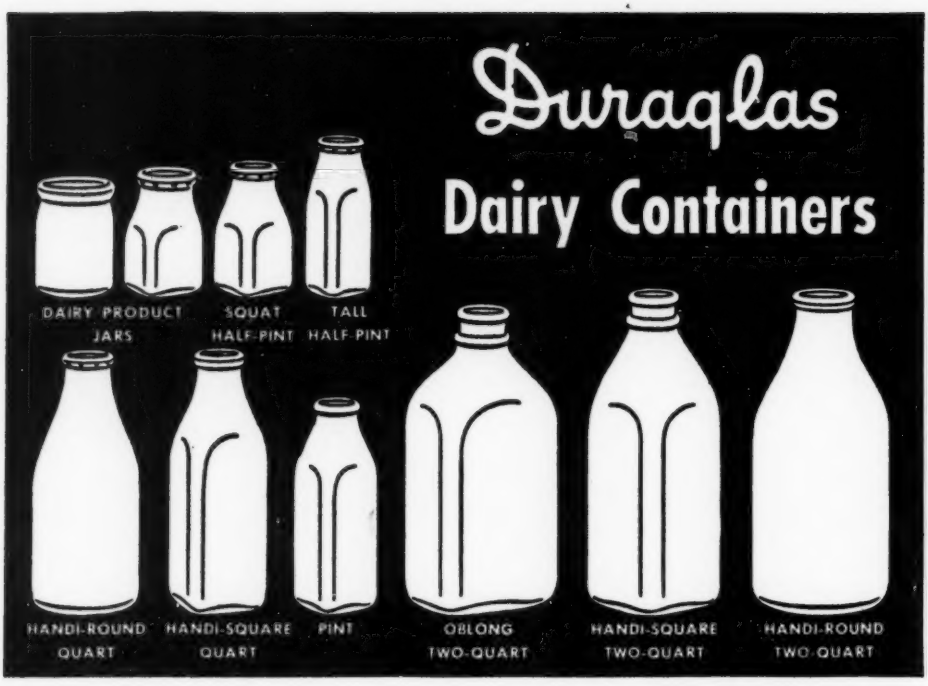
A Comparison of Penicillin, Streptomycin and Sulfanilamide for Improving the Fertility of Semen from Bulls of Low Fertility. JOHN O. ALMQUIST 819

Proceedings of the Forty-sixth Annual Meeting, Knoxville, Tennessee 823

Abstracts of Literature A63

Vol. XXXIV, No. 8, August, 1951

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION



...pass the test for true economy

To determine the cost of dairy containers, look beyond their first cost.

High-quality, returnable glass bottles are unequaled for keeping container costs low. That's why Duraglas bottles are so carefully controlled in manufacture to assure high quality and long life. You can depend on them to deliver dependably the high trippage that means *true economy.*

ACL—Each bottle can be a "talking salesman" for the dairy with Applied Color Lettering designs. Stock designs are always available, and our artists are ready to develop special designs.

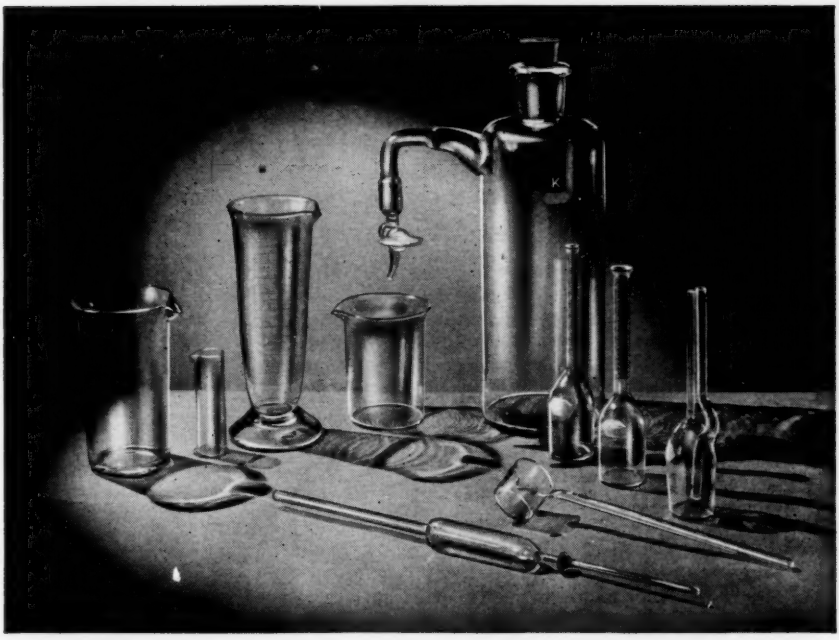
FOR ALL YOUR DAIRY NEEDS, SPECIFY

Duraglas Dairy Containers

TRADE MARK REG. U.S. PAT. OFF.

OWENS-ILLINOIS GLASS COMPANY • TOLEDO 1, OHIO • BRANCHES IN PRINCIPAL CITIES

Your advertisement is being read in every State and in 45 Foreign Countries



Glassware for your laboratory

. . GET IT FROM CHERRY-BURRELL!

Complete stocks of glassware for all standard types of milk, cream and other dairy laboratory tests and analyses are maintained by Cherry-Burrell Branches and Associate Distributors. Special glassware is quickly available. For prompt information and service, call or write our nearest office.

CHERRY-BURRELL CORPORATION
General Sales and Executive Office:
427 W. Randolph Street, Chicago 6, Ill.
Milk and Food Plant Equipment and Supplies
FACTORIES, WAREHOUSES, BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 36 CITIES

trendmaker in a great industry

Your advertisement is being read in every State and in 45 Foreign Countries

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

F. E. NELSON, *Editor*
Ames, Iowa

Associate Editors

F. J. DOAN State College, Penn.	P. R. ELLIKER Corvallis, Ore.	I. A. GOULD Columbus, Ohio	H. A. HERMAN Columbia, Mo.
L. A. MOORE Beltsville, Maryland	W. V. PRICE Madison, Wis.	I. W. RUPEL College Station, Tex.	G. H. WISE Raleigh, N. C.

Committee on Journal Management

G. H. WISE, *Chairman*

J. K. LOOSLI — F. E. NELSON, *ex officio*
W. V. PRICE — P. R. ELLSWORTH, *ex officio*

Subscriptions. Price, $10.00 per volume in North and South America; $10.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising copy should be mailed to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio. Advertising plates or cuts should be mailed direct to the Business Press, Inc., N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to P. R. Ellsworth at the address above stated.

OFFICERS OF THE ASSOCIATION

R. B. BECKER, *President*
Gainesville, Fla.

H. A. BENDIXEN, *Vice-President*
Pullman, Wash.

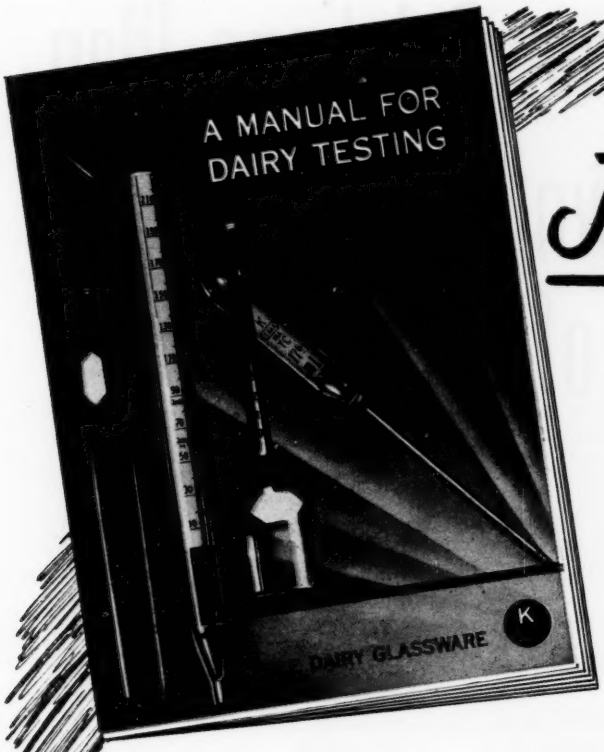
P. R. ELLSWORTH, *Sec.-Treas.*
Columbus, Ohio

F. E. NELSON, *Journal Editor*
Ames, Iowa

DIRECTORS

F. J. ARNOLD Ames, Iowa	P. R. ELLIKER Corvallis, Ore.	J. H. ERB Columbus, Ohio	H. B. HENDERSON Athens, Ga.
L. A. MOORE Beltsville, Maryland	G. M. TROUT East Lansing, Mich.		C. W. TURNER Columbia, Mo.

Entered as second-class matter April 13, 1934, at the postoffice at Lancaster, Pa. under the act of March 3, 1879.



Just out!

4th edition of the KIMBLE MANUAL

—Tells how to test dairy products accurately

If you sample or test dairy products you'll want the new 4th edition of the famous Kimble Dairy Manual.

Specially prepared after months of research, the Kimble Manual for Dairy Testing gives information on the Babcock test; how to test milk, cream, butter, cheese, ice cream; tests for milk with sugar or chocolate; eight others. Also pertinent tables and a listing of Federal and State Standards for dairy products.

KIMBLE GLASS TOLEDO 1, OHIO
Division of Owens-Illinois Glass Company

K

Send for your free copy today

MAIL THIS →

Kimble Glass
Division of Owens-Illinois Glass Company
Toledo 1, Ohio
Please send me a *free* copy of the new Kimble Dairy Manual.
Name_____
Address_____
City_____ State_____

Your advertisement is being read in every State and in 45 Foreign Countries

B-K® Patron Relations Plan cuts down number of trouble calls

writes Mr. John M. Good, fieldman for Lamar Creamery, Paris, Texas.

A fieldman for a dairy in a rapidly-expanding area like Paris, Texas, is a *very* busy man. He has many duties more important than "housekeeping" calls on erring producers.

The P R Plan *automatically* makes periodic sanitation calls on all patrons. That's why this plan is so valuable to fieldmen.

The Plan is all ready to use! It is a coordinated milk-improvement program, carefully designed to help fieldmen and sanitarians impress upon their producers the importance of day to day, year 'round "quality thinking", whether a sanitarian or fieldman calls personally or not.

The Patron Relations Plan consists of 3 *free* services, provided in the interests of the dairy industry by the makers of B-K cleansers and sanitizers.

1. "TIMELY TIPS"—a monthly bulletin for producers on seasonal dairy problems.

2. MOVING PICTURES and sound-slide films on quality milk production, for showing at patron meetings.

3. THE FREE ASSISTANCE of trained field representatives in planning and conducting patron meetings.

Fieldmen and plant owners everywhere tell about the effectiveness of this B-K Patron Relations Plan in improving milk quality.

Health officers and milk sanitarians who would like to receive the Plan regularly are invited to write: Dept. 28, Pennsylvania Salt Manufacturing Company—*East:* Philadelphia 7, Pa. *West:* Woolsey Building, 2168 Shattuck Ave., Berkeley 4, Calif.

PENNSALT CHEMICALS

for Industry • Agriculture • Health • Home

PROGRESSIVE CHEMISTRY FOR OVER A CENTURY

Your advertisement is being read in every State and in 45 Foreign Countries

"That's what I need—a compact unit to go with my ice builder."
"That 4 cylinder job will just fit our space and handle peak cooling and storage."
Two cyl. 5 x 5 Marine Type Self-Contained Unit.
Four cyl. 3½ x 3½ Type K Ammonia Compressor.
Two cyl. 8 x 8 Vertical Type Compressor.
"That's my next buy for sub-zero freezing work."
"Another 8 x 8 would take care of our second stage load."
Six cyl. 6¾ x 5 Type K Booster.

Look ahead with CP Refrigeration

From the CP "Balanced Line" you'll get —not just a compressor—but *exactly* the unit *you* need, to do *your* refrigeration job . . . large or small . . . with efficiency and economy assured by CP's more than 60 years of refrigeration pioneering and leadership. You're *right* when you choose CP.

THE CREAMERY PACKAGE MFG. COMPANY
General and Export Offices: 1243 W. Washington Blvd., Chicago 7, Illinois

Your advertisement is being read in every State and in 45 Foreign Countries

FILTER FACTS for DAIRY SANITARIANS

THE RIGHT SIZE Filter Disk for the Strainer is Important

Let's face facts, many farmers disregard the *size* of the filter disks they buy, in their efforts to get by the cheapest way possible. Result: many filter disks are too small for the strainer. By-pass of *unfiltered* milk may result, carrying sediment that may even cause rejection at the plant—a loss to the plant, producer and the hauler.

RAPID-FLO Fibre-Bonded

Trademark of Johnson & Johnson

Filter Disks are made in sizes to fit all strainers in general use

J & J field research has noted this situation. Consistent with Johnson & Johnson policy of providing every possible aid to the dairy industry, they make Rapid-Flo FIBRE-BONDED filter disks (all types—no gauze facing, single gauze faced and double gauze faced) in sizes to meet all general conditions. To help plants gain cooperation from producers, J & J has developed a simple check-up program.

FILTER-STRAINER CHECK-UP

USE THE *RAPID-FLO* FILTER STRAINER CHECK-UP TO ASSURE CORRECT SIZE AS WELL AS TYPE

Your J & J Filter Products Division Field Service representative will cooperate fully in providing you with sufficient cards for your producers, and explain their use. Contact him or write the factory for details.

FILTER PRODUCTS DIVISION

Johnson & Johnson

4949 West 65th Street · Chicago 38, Illinois

JOURNAL OF DAIRY SCIENCE

VOLUME XXXIV AUGUST, 1951 NUMBER 8

STUDIES ON OXIDIZED MILK FAT. I. OBSERVATIONS ON THE CHEMICAL PROPERTIES OF THE VOLATILE FLAVOR MATERIAL FROM OXIDIZED MILK FAT[1]

MARK KEENEY[2] AND F. J. DOAN

Department of Dairy Husbandry, The Pennsylvania Agricultural Experiment Station, State College

Many dairy products, especially those which are placed in storage before consumption, are susceptible to off-flavor development due to the oxidative deterioration of the milk fat. Very little information is available on the chemical nature of the characteristic flavor compounds present in oxidized milk fat. The present study involved isolation and chemical characterization of some of the volatile material from oxidized milk fat with particular attention being given to the fractions containing the characteristic flavor and odor compounds.

EXPERIMENTAL

Preparation of oxidized milk fat. Two kg. of milk fat were prepared from unsalted, sweet cream butter, which had been in frozen storage for 4 mo. The butter was melted and washed with hot tap water (71° C.) by passing the butter and water through a centrifugal cream separator until a clear, curd-free oil was obtained. The milk fat was allowed to stand in a loosely stoppered pyrex Erlenmeyer flask in a place in the laboratory where it would be exposed to the maximum amount of diffuse sunlight. Within a week, the fat had developed a fruity odor reminiscent of fresh strawberries. The yellow color gradually was dissipated, and at the end of 6 wk., the fat was completely bleached and almost water clear. During the later stages of the bleaching period, the fruity odor disappeared and a typical stale fat odor appeared. Two wk. after the fat had become bleached it had a strong penetrating odor of oxidized fat. At this time, various methods for removing the flavor substances from the fat were tried.

Vacuum distillation of oxidized fat. In preliminary experiments on methods for removing the flavor substances from the oxidized fat, it was observed that during vacuum steam distillation of the fat there was a diffusion of oxidized flavor material from the steam-condensate receiver, immersed in an ice bath, to a

Received for publication Feb. 5, 1951.

[1] Authorized for publication Feb. 16, 1951, as paper no. 1654 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Present address: Dairy Department, University of Maryland, College Park.

Copyright, 1951, by the AMERICAN DAIRY SCIENCE ASSOCIATION.

second receiver immersed in an alcohol-dry ice bath. The apparent diffusion of the flavor material into this dry ice trap suggested that direct vacuum distillation of the fat, without employing steam, would be of value.

Approximately 400 g. of fat were placed in a 500-ml. distilling flask and, to facilitate distillation, about 10 g. of finely broken non-glazed porcelain were added to the fat. A 2-ml., flat bottom, glass vial was fitted loosely over the end of the side arm of the 500-ml. distilling flask. The side arm of this distilling flask led directly into a 50-ml. distilling flask immersed in an alcohol-dry ice bath. The side arm of this receiving flask was connected by heavy gauge rubber tubing to a few turns of copper tubing, immersed in alcohol-dry ice, which was in turn connected to a Cenco Hyvac pump. The rubber tubing between the pump and copper coil contained a ground glass stopcock, while the tubing between the copper coil and receiving flask contained a two-way ground glass stopcock. The distilling and receiving flasks were connected by a tightly fitted rubber stopper and the distilling flask was fitted with a solid rubber stopper to complete the system.

During distillation, the volatile material collected for study was entrained in the side arm of the 500-ml. distilling flask or in the 2-ml. vial fitted over the end of the side arm.

A cubical asbestos oven (5.5 in. per side) was assembled around the distilling flask, fitted with a thermometer and a Bunsen burner placed beneath as a source of heat. The bottom of the distilling flask was about 0.5 in. from the bottom of the oven, which was loosely assembled and had a sufficiently large opening, where the neck of the flask emerged from the top of the oven, to permit a rapid escape of hot air.

With the distillation assembly completed, the vacuum pump was started, and the stopcocks were turned to the proper position to evacuate the system. The burner under the oven was lighted and regulated so that there was a slow rise in temperature inside the oven. The temperature of the oven reached 160 to 180° C. in about 15 min. and was maintained in this range during the 30-min. distillation period with a pressure in the system of 0.5 to 1.0 mm. of mercury. At the end of the distillation period, the burner under the oven was turned off and the system carefully vented. The receiving flask was dismantled and the distillate in the vial and side arm was collected. This distillation procedure yielded 1 g. of volatile material from 1,200 g. of the oxidized fat. The same distillation procedure applied to 400 g. of fresh milk fat yielded only about 50 mg. of distillate. The odor of the volatile material from the oxidized fat was extremely strong and lingering, suggestive of the oxidized fat from which it was obtained. The odor of the small amount of distillate from the fresh fat was relatively mild and not characteristic of oxidized fat.

Chemical properties of volatile material. The distillate obtained from the oxidized fat was a yellow oily liquid at room temperature. Preliminary qualitative tests on the distillate gave the following information: acid to moist litmus paper; reacted with 3 per cent $FeCl_3$ reagent to give a purple red color; decolorized bromine in carbon tetrachloride; reduced neutral $KMnO_4$ at room tem-

perature immediately; did not appear to react with alkaline iodine in KI; the bulk of the material was insoluble in water and soluble in ethanol and ethyl ether.

The preliminary observations suggested that a further fractionation of the distillate would be of value. Consequently, a 500-mg. portion of the material was dissolved in 25 ml. of ethyl ether and washed with two 20-ml. portions of 5 per cent K_2CO_3 solution in a 100-ml. separatory funnel. Then it was successively washed with 15 ml. of water (containing two drops of concentrated HCl) and 10 ml. of water. The ether was evaporated on a warm water bath to yield about 300 mg. of a light yellow-colored oily liquid. This material will be referred to in future discussion as the neutral fraction.

The carbonate washings from the distillate were made acidic by the dropwise addition of concentrated HCl. The acid solution was extracted with four 20-ml. portions of ether. The ether was evaporated to yield about 100 mg. of dark brown oily material; hereafter this will be referred to as the acid fraction.

Characteristics of neutral fraction: Retained typical odor of original distillate; $FeCl_3$ test, negative; reacted with 2,4-dinitrophenylhydrazine reagent to give a red precipitate; reduced neutral $KMnO_4$ immediately; remained liquid at $-5°$ C.; reduced hot Fehling's solution; refractive index 1.458 at 25° C.; attempt at boiling point determination unsuccessful as heating of fraction in open capillary tube, immersed in oil bath, resulted in progressive browning up to 170° C.

Characteristics of acid fraction: Faint odor similar to caproic or caprylic acid; solid at $-5°$ C.; $FeCl_3$ test positive; a slow poor-yield reaction with 2,4-dinitrophenylhydrazine reagent; acid to litmus paper; bulk of material insoluble in water; fraction seemed extremely unstable and prone to browning when exposed to air and heat.

All volatile fractions were stored in tightly stoppered glass vials at $-5°$ C. and removed from cold storage only long enough to obtain samples for testing.

Significant role of carbonyl groups in odor and flavor. When a small amount of the neutral fraction was reacted with an excess of semicarbizide hydrochloride, the reaction mixture lost the typical odor of oxidized fat. Hydrolysis of the crude semicarbazones with 5 per cent HCl resulted in reappearance of the odor. This illustrates the important contribution of carbonyl compounds to the oxidized fat odor of the neutral fraction.

Small quantities of the neutral and acid fractions were added to milk and the samples examined for odor and flavor qualities. Less than 1 ppm. of the neutral fraction imparted a strong oily or oxidized flavor to milk. On the other hand, several ppm. of the acid fraction were added to milk with no noticeable effect on the flavor.

Preparation of 2,4-dinitrophenylhydrazone from neutral fraction. After the above observations had been made, very little of the acid fraction remained; however, enough of the neutral fraction was on hand for an attempt at derivative preparation. Because of the apparent contribution of carbonyl compounds to the typical odor of the neutral fraction, the preparation of carbonyl derivatives from this fraction was attempted in order to gain information on the structure

of these compounds. The neutral fraction was reacted with 2,4-dinitrophenylhydrazine as follows: Thirty ml. of 95 per cent ethanol were added to 170 mg. of 2,4-dinitrophenylhydrazine in a 50-ml. round botton flask. After the addition of 20 drops of concentrated HCl, the mixture was heated on a steam bath to dissolve all the reagent. A mixture of 95 mg. of neutral fraction dissolved in 7 ml. of ethanol was added to the hot solution of the reagent. The reaction mixture was brought to the boiling point and immediately removed from the steam bath and allowed to cool slowly to room temperature; a small amount of red precipitate began to form. After 2 hr., the mixture was placed in the freezing compartment of a refrigerator (−5° C.) for a day. It then was transferred to a 50-ml. centrifuge tube, centrifuged a few minutes at 1,500 rpm. and the clear supernatant liquid decanted from the precipitate. An attempt was made to redissolve the precipitate in 25 ml. of hot ethanol. This resulted in solution of most of it; however, a small amount of insoluble red oil remained. The hot solution was decanted from this heavier oil and, after holding for 3 days at −5° C., it was centrifuged again. The supernatant liquid was decanted and the precipitate dried under vacuum. This precipitate (about 30 mg.) was a red amorphous powder, melting at 90 to 95° C.

Further purification of the powder was accomplished by chromatography. It was dissolved in 200 ml. of ethyl ether and passed through an adsorption column consisting of a 70-cm. section of 10-mm. glass tubing packed with Fisher adsorption alumina (80 to 200 mesh), glass wool being used as a plug at the bottom. Slow passage of the ether solution through the column gave a reddish-brown band about 20 cm. from the top of the column. This band slowly widened and moved down the column. By the time all of the 200 ml. of ether solution had been passed through the column, the band was about 6 cm. wide, 50 cm. from the top of the column. The band was eluted with ether, which was evaporated from the eluate giving 15 mg. of red powder. Three crystallizations of the powder from 5 ml. of 95 per cent ethanol gave 12 mg. of fine red needles, melting at 105 to 107° C. The final two crystallizations of this material produced no change in the melting point.

During chromatography some material in the original ether solution was not adsorbed. Evaporation of the ether from this material gave an orange oil of such quantity and character that further study of it was precluded.

Elemental analysis of the crystalline 2,4-dinitrophenylhydrazone (m.p. 105 to 107° C.) gave 56.84 per cent carbon, 6.46 per cent hydrogen and 17.24 per cent nitrogen. The calculated analysis for the 2,4-dinitrophenylhydrazone of a mono-ethylenic nine-carbon mono-carbonyl compound ($C_9H_{16}O$) is 56.24 per cent carbon, 6.29 per cent hydrogen and 17.49 per cent nitrogen.

It is assumed that the compound giving the above derivative had a carbon-to-carbon double bond conjugated with the carbonyl double bond. Aliphatic saturated carbonyl compounds invariably give yellow-colored 2,4-dinitrophenylhydrazones, whereas α, β-unsaturated carbonyl compounds usually give red-colored 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazone of 2-nonenal melts at 126° C. (4). This suggests that the derivative prepared was from an

unsaturated ketone but the negative reaction of the unfractionated volatile distillate with iodine in alkaline potassium iodide further suggests that it was not a methyl ketone.[3] This leaves one of the five isomeric nine-carbon α, β-unsaturated ketones with the oxygen in the three, four or five position as the tentative structure assigned to the compound giving the derivative. Since there appears to be no information available on these unsaturated ketones, no good choice can be made between the five possibilities.

DISCUSSION

It is recognized that the milk fat used in this study was subjected to more extreme oxidizing conditions than normally would be encountered in dairy products. The use of relatively high temperatures (160 to 180° C.) for distillation probably resulted in further deterioration of the oxidized fat. The question of whether or not a study of milk fat vigorously oxidized by air, sunlight and heat can be useful in clarifying the practical problems encountered when milk fat is oxidized under less strenuous conditions remains to be answered. This point is worthy of further study and will be reported on in a subsequent paper in this series. Regardless of any theoretical objections, the odor and flavor fidelity of the neutral fraction in simulating the organoleptic characteristics of oxidized fat suggested that a continuation of the present line of approach would be worthwhile.

According to Lea (1), atmospheric oxidation of unsaturated fats has been shown to give rise to peroxy-, oxy-, hydroxy- and keto-glycerides or acids, hydrogen peroxide, carbon monoxide and carbon dioxide, dicarboxylic acids, acrolein, epihydrin aldehyde, glyceric-aldehydes and acids and almost the complete series of volatile aliphatic mono basic acids and aldehydes from formic to nonanoic. In many cases, conclusive identification of some of the above compounds in autoxidized fats has not been accomplished. Scala (3), in an early study of oxidized fats, concluded that the volatile neutral fractions from such fats contain butanal, heptanal and nonanal and, furthermore, that heptanal and nonanal are responsible for the characteristic odor of such fats. Some questions may be raised as to the validity of Scala's conclusions since he chemically oxidized his neutral fraction to acids, identified the acids and concluded that the precursors of the acids were the corresponding aldehydes. Recently, Martin *et al.* (2) have presented evidence to indicate that 2-heptenal is present in oxidized soybean oil. Swift *et al.* (5) have reported the identification of hexanal, 2,4-decadienal and 2-octenal from the steam distillate of oxidized cottonseed oil. Van der Waarden (6), after a study of flavor deteriorated cold storage butter, has postulated that methyl ketones and aliphatic unsaturated C_4 to C_8 aldehydes (with no double bond conjugation with the carbonyl bond) are probable contributors to the off-flavors.

[3] A reviewer of this paper has logically suggested that crystallization of this compound from a new solvent may have resulted in an increase in the melting point. In paper II of this series a derivative is reported with the same elemental composition, melting at 109–110° C., prepared from a fraction exhibiting a negative Fehling's test.

In the present study, it was observed that the predominant odor and flavor compounds from oxidized milk fat were in the volatile neutral fraction, whereas the volatile acid fraction was an insignificant contributor to the odor and flavor of the fat.

The chemical properties of the neutral fraction indicated unsaturation and the presence of carbonyl compounds. Reaction of the neutral fraction with Fehling's solution indicated the presence of aldehydes; however, no satisfactory aldehyde derivatives were obtained with 2,4-dinitrophenylhydrazine reagent. The carbonyl derivative prepared from the fraction indicated the presence of an unsaturated ketone and the possible structures for this ketone have been discussed.

CONCLUSIONS

The vacuum distillation of oxidized milk fat yielded material with the characteristic flavor and odor of the oxidized fat.

The significance of carbonyl compounds as contributors to the characteristic odor of oxidized fat and the relative insignificance of the volatile acids as contributors to the odor has been demonstrated.

The addition of the volatile neutral material to milk was capable of imparting an oxidized flavor to the milk when present in concentrations of less than 1 ppm. This flavor was similar but not identical to that commonly encountered in oxidized milk on the market.

A 2,4-dinitrophenylhydrazone was prepared from the volatile neutral fraction, with an empirical formula $C_9H_{16}O$ indicated for the parent compound. Theoretical considerations indicated that the compound was an α, β-unsaturated ketone with the oxygen in the three, four or five position.

ACKNOWLEDGMENTS

The authors are indebted to the Microchemical Department of Hoffmann-La Roche, Inc., Nutley, N. J., under the direction of A. Steyermark, for the performance of elemental microanalyses in connection with this research.

REFERENCES

(1) Lea, C. H. *Rancidity in Edible Fats.* Chemical Publishing Co., New York. 1939.

(2) Martin, C. J., Schepartz, A. I., and Daubert, B. F. Flavor Reversion in Soybean Oil. IV. Isolation of Reversion Compounds in Soybean Oil. J. Am. Oil Chem. Soc., **25**: 113–117. 1948.

(3) Scala, A. Su di alcune sostauze volatili e non volatili che si formano nell'irrancidimento dei grassi. Gaz. Chem. Ital., **38i**: 307–327. 1908.

(4) Scanlon, J. T., and Swern, D. Action of Lead Tetracetate upon Hydroxylated Fat Acids and Related Compounds. II. Hydroxylated Ricinoleic Acid and Castor Oil. J. Am. Chem. Soc., **62**: 2309–2311. 1940.

(5) Swift, C. E., O'Connor, R. T., Brown, L. E., and Dollear, F. G. The Aldehydes Produced during the Autoxidation of Cottonseed Oil. J. Am. Oil Chem. Soc., **26**: 297–300. 1949.

(6) Van der Waarden, M. Chemical Processes Underlying the Deterioration of the Flavor of Butter in Cold Storage. Research in Holland, pp. 155–206. 1947. (Cited in Chem. Abs., **42**: 5129b. 1948.)

STUDIES ON OXIDIZED MILK FAT. II. PREPARATION OF 2,4-DINITROPHENYLHYDRAZONES FROM THE VOLATILE MATERIAL FROM OXIDIZED MILK FAT[1]

MARK KEENEY[2] AND F. J. DOAN

Department of Dairy Husbandry, The Pennsylvania Agricultural Experiment Station, State College

In a previous paper (3), it was reported that the vacuum distillation of oxidized milk fat yielded material containing a high concentration of the characteristic odor compounds of oxidized fat. The preparation of 2,4-dinitrophenylhydrazones showed promise as a means of obtaining identification data on the volatile carbonyl compounds which seemed to be the predominant odor and flavor compounds from the oxidized fat. This paper reports a continuation of the chemical study of the volatile flavor material from oxidized milk fat.

EXPERIMENTAL

Ten gal. of fresh raw cream (40 per cent fat) were diluted with four volumes of tap water and reseparated into cream (40 per cent fat). This water-washing procedure was repeated once and the washed cream cooled and held at 13° C. for 4 hr. before churning into butter. The butter was passed through a cream separator after mixing with hot tap water (71° C.) until a clear, curd-free oil was obtained from the centrifuge. This fat had a bland flavor. After standing at room temperature for 48 hr., the fat was filtered through cheese cloth by gravity and gave about 8 lb. of solid fat and 16 lb. of liquid fat. Three kg. of the precipitate or solid fat were placed in a loosely stoppered 4-l. pyrex Erlenmeyer flask on a hot plate exposed to northwest sunlight. The temperature of this fat was maintained at about 60° C. on the hot plate which was lined with asbestos boards to prevent excessive localized heating of the fat. Three kg. of the liquid fat, from the above filtration through cheese cloth, were placed in a loosely stoppered Erlenmeyer flask and similarly exposed to sunlight, but maintained at room temperature. The remainder of the fat, solid and liquid, was placed in cold storage (5° C.) for subsequent control experiments.

The separation of the whole milk fat by the above procedure resulted in a liquid fat fraction which was considerably deeper in yellow color than the higher-melting solid fraction. After 4 wk. the fat held at 60° C. was completely bleached. The fat held at room temperature was bleached in 10 wk.

After 10 wk. the fat stored at 60° C. was distilled at about 0.5 mm. of mercury from an oven (160 to 180° C.), in the manner described previously (3), using successive 400-g. portions. Three kg. of this fat gave about 3 g. of volatile material.

Received for publication Feb. 5, 1951.

[1] Authorized for publication on February 16, 1951, as paper no. 1655 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Present address: Dairy Department, University of Maryland, College Park.

The volatile material was dissolved in 100 ml. of ether and washed twice with 50-ml. portions of 2.5 per cent K_2CO_3. The ether was washed free of residual carbonate with very dilute HCl and water and, after evaporation of the ether the yield of neutral fraction, amounting to about 2 g. exhibited an extreme odor of oxidized fat.

Fractional distillation of volatile neutral material. The volatile neutral material was fractionally distilled from a 10-ml. round-bottom flask through a 23 cm. section of 6 mm. glass tubing packed loosely with a piece of twisted Nichrome wire. The receiver was a 10-ml. distilling flask immersed in alcohol-dry ice. During distillation the pressure in the system was about 0.5 mm. of mercury, maintained with a Hyvac pump, and once every hour the system was vented and the receiver changed. After each change of receiver, vacuum again was applied to the system and the distillation continued. Heat was applied from a beaker of boiling water below the pot. During most of the distillation period, "flooding" occurred in the bottom half of the column. Data pertaining to fractions obtained from the distillation are given in table 1.

TABLE 1

Fractional distillation of neutral material

Fraction no.[a]	Pot temp.	Approx. yield	n_D^{25}	Fehling's
	(*C.*)	(*mg.*)		
1	25°	150	1.4260	negative
2	80–100°	100	1.4390	negative
3	80–100°	100	1.4420	negative
4	80–100°	100	1.4445	negative
5	80–100°	20	1.4425	negative
6	80–100°	100	1.4490	positive
7	80–100°	50	1.4430	positive
8	80–100°	50	1.4430	positive
Pot residue		600	1.4695	positive
Original charge		1800	1.4630	positive

[a] Each fraction represents operation of the still for 1 hr. Still operated for total of 8 hr.

The yield figures in table 1 are only approximate because some loss of material was involved in manipulation of the equipment. Refractive index readings were made with an Abbe Spencer refractometer fitted with Amici prisms. Fehling's test was conducted by diluting standard Munson-Walker reagent with three volumes of water. To 2 ml. of diluted reagent 5 to 10 mg. of test material were added in a 5-ml. test tube. After standing for 15 min. at room temperature, the tube was immersed in a boiling water bath for 15 min. If no reduction of the solution appeared after this time, the test was considered negative. Due to the relative water insolubility of the fractions, some question may be raised concerning the validity of the procedure. However, since all of the fractions seemed to have the same solubility characteristics and yet reacted differently, it was felt that this procedure for the Fehling's test is justified.

All of the fractions in table 1 reduced neutral $KMnO_4$ immediately at room temperature, and gave red-orange precipitates with 2,4-dinitrophenylhydrazine

reagent. The whole neutral material before fractionation gave a questionable reaction with alkaline iodine in KI. Some precipitate formed but it did not appear to be iodoform. The first five fractions in table 1 gave no precipitate with this reagent. In the application of the iodoform test, dioxane was used to insure solubilization of the test material as recommended by Shriner and Fuson (10).

All the volatile fractions (table 1) were colorless liquids, the first five of which had rather sharp penetrating odors, while fractions 6, 7, 8 and the pot residue exhibited "oily" odors without the sharpness of the more volatile fractions. Regardless of minor differences in the odor of the various fractions, they all had pronounced odors characteristic of oxidized fat.

The pot residue had become slightly darker in color than the original whole neutral fraction and, after standing overnight at room temperature, a slight amount of white precipitate was observed. This suggested that cold solvent crystallization could be employed to advantage for separating one or more constituents from this residue. The pot residue was dissolved in 5 ml. of 95 per cent ethanol and the solution was allowed to stand for 1 wk. at $-18°$ C., after which it was filtered by gravity at $-18°$ C. to yield a small amount of precipitate. The bulk of the pot residue was retained in the filtrate. The precipitate was washed from the filter paper with a small amount of ethanol which subsequently was evaporated under reduced pressure to yield about 100 mg. of a yellow-brown oily liquid at room temperature. This material was a solid at 4° C., n_D^{25} 1.4670, and had an odor suggestive of oxidized fat but not the irritating odor of some of the more volatile portions of the neutral fraction.

Preparation of 2,4-dinitrophenylhydrazones from neutral fractions. Both the precipitate and filtrate from the solvent fractionation of the pot residue, as well as fractions 1 and 2, 3, 4 (combined) from the fractional distillation of the neutral fraction were reacted with 2,4-dinitrophenylhydrazine. These reactions were conducted by dissolving the fraction (50 to 300 mg.) in 10 ml. of ethanol and adding 2,4-dinitrophenylhydrazine (100 to 300 mg.) dissolved in 10 to 15 drops of concentrated H_2SO_4. The reaction mixture was allowed to stand for 1 hr. at room temperature, after which water was added to the cloud point. After standing 4 to 5 hr. at 4° C., the reaction mixture was centrifuged at 1,500 rpm., the clear supernatant liquid decanted and the precipitate dissolved in 5 to 15 ml. of hot ethanol. This was stored at 4° C. overnight, after which water was added to complete the precipitation, the precipitated solution centrifuged, the supernatant decanted and the precipitate dried under vacuum. The dry precipitate was chromatographed on a column (2.5×20 cm.), packed with a mixture of 40 g. of Fisher adsorption alumina (80 to 200 mesh) and 20 g. of Volclay bentonite (80 to 100 mesh). Various solvents were used during chromatography and a slight amount of suction was used to force a slow passage of solvent through the column. The receiver below the column was immersed in an alcohol-dry ice bath to prevent excessive evaporation of solvent in the receiver and lower part of the column. Data pertaining to the preparation and chromatography of 2,4-dinitrophenylhydrazones from the various fractions shown in table 1 are summarized in table 2.

TABLE 2

2,4-Dinitrophenylhydrazones from neutral fractions

Fraction from table 1		Chromatographic solvents[a]	Characteristics of 2,4-Dinitrophenylhydrazones										Formula of calculated parent compound
							Analysis per cent						
							Found			Calculated			
No.	Wt. used		Wt. major Hydrazone	m.p.	Color	Wave length abs. max.[b]	C	H	N	C	H	N	
	(*mg.*)		(*mg.*)	(° *C.*)		(mμ)							
1	50	pet. ether ethyl ether	5	83–5	orange	363–4			19.24			19.17	$C_7H_{12}O$
2, 3, 4 combined	100	pet. ether ethyl ether	12	109–10	red-orange	373–4	56.35	6.69	17.08	56.24	6.29	17.49	$C_9H_{16}O$
		Pet. ether ethyl ether 5% methanol in ethyl ether	20	146	yellow		42.75	3.79	25.14	42.86	3.60	24.99	CH_3CHO
Pot residue ppt.	70	ethyl ether 5% methanol in ethyl ether	30	147	orange	356–7	42.28	3.50	22.92	42.52 42.86	3.97 3.20	22.04 22.22	$C_3H_6O_2$ $C_3H_4O_2$
Pot residue filtrate	300	pet. ether 10% ethyl ether in pet. ether	40	115–16	red-orange	377–8	59.63	6.68	15.36	59.98 59.65	6.71 7.22	15.54 15.46	$C_{12}H_{20}O$ $C_{12}H_{22}O$

[a] Solvents listed in order of use. First listed solvent used for adsorption of hydrazones on column. Next listed solvents used for developing and eluting the band. Solvents were changed gradually during development and elution.

[b] In 95 per cent ethanol.

The solvents used for chromatography were made as dry and pure as practically possible before use. The ethyl ether was distilled from sodium and stored over anhydrous Na_2SO_4. The petroleum ether was distilled and the fraction boiling between 38 and 42° C. was stored over anhydrous Na_2SO_4. The methanol was synthetic Columbian Spirits distilled twice from zinc and NaOH.

The yield figures for the hydrazones in table 2 represent the weight of the derivative after chromatography and recrystallization from ethanol to constant melting point. During preparation of the reported derivatives, it was observed that small amounts of other 2,4-dinitrophenylhydrazones were present. The only derivatives reported are the ones obtained in sufficient quantity for elemental analysis. The reactions did not appear to be quantitative, indicating that the fractions contained substances other than carbonyl compounds or that the carbonyl compounds did not react quantitatively under the conditions used.

Some of the fractions yielded small quantities of red oil when reacted with 2,4-dinitrophenylhydrazine. These red oils were insoluble in hot ethanol and soluble in nitrobenzene. The low melting points of these oils and their solubility characteristics precluded their purification for analysis in the present study. These oils seemed to be true hydrazones, as evidenced by red color development when added to alcoholic NaOH (7).

The absorption spectra of the hydrazones were obtained by using a Beckman Model DU Spectrophotometer with the samples dissolved in 95 per cent ethanol. The ethanol was prepared by two distillations of 95 per cent ethanol from zinc and NaOH. The concentrations of the hydrazones in ethanol were such that the optical densities of the solutions at the wave lengths of maximum absorption were between 0.2 and 0.6.

Odor and flavor characteristics of neutral fractions. During the preparation of the 2,4-dinitrophenylhydrazones from various portions of the neutral fraction, it was noticed that the reaction mixtures lost the typical odor of oxidized fat. This confirmed a previous observation on the significant role of carbonyl groups in the odor of oxidized milk fat (3).

The addition of the volatile neutral fraction from oxidized fat and various parts of it to milk imparted distinct off-flavors to the milk. The exact character of the off-flavors is rather difficult to describe, but the whole neutral fraction, when added to milk, seemed to closely simulate the flavor of oxidized milk as it is commonly encountered. The more volatile parts of the neutral fraction, which appeared to contain unsaturated ketones, did not simulate this flavor as closely as the whole neutral fraction. The concentration of these neutral materials necessary in milk to produce noticeable off flavors was on the order of one part in five to ten million parts of milk.

No observations were made on the volatile acids from this lot of fat because the carbonate washings from the volatile material were lost through a laboratory accident.

DISCUSSION

The data presented in table 2 indicate that the hydrazone (m.p. 83–85° C.) from fraction 1 (table 1) was derived from a seven-carbon ketone. The negative

iodoform test of the fraction would place the carbonyl group in the three or four position, assuming the ketone to be straight-chained. The reaction of the fraction with neutral $KMnO_4$ and the refractive index (1.4260) indicate unsaturation. The absorption maximum of the hydrazone at 363 to 364 mμ indicated that there were no double bonds conjugated with the carbonyl group. The above information would suggest that the best postulated structure for the ketone is 6-heptene-3-one, 5-heptene-3-one or 1-heptene-4-one. Since the 2,4-dinitrophenylhydrazones of these three ketones have not been reported, no good choice can be made among them.

The hydrazone (m.p. 109 to 110° C.) derived from the combined fractions 2, 3, 4 (table 1) was evidently that of a nine-carbon ketone with a carbon-to-carbon double bond conjugated with the carbonyl group. The red color of the derivative as well as the wave length of absorption maximum (373 to 374 mμ) indicated conjugation. The negative iodoform test of the fraction indicated that it was not a methyl ketone. This derivative was similar or identical with the one isolated from a previous batch of fat (3).

The other hydrazone (m.p. 146° C.) derived from the combined fractions 2, 3, 4 (table 1) is obviously that of acetaldehyde. This derivative is the result of a decomposition reaction. Under the conditions of the reaction for the preparation of the hydrazone, something in the fraction decomposed to acetaldehyde or the ethanol used in the reaction was oxidized to acetaldehyde. Since this is the only instance in which this hydrazone was obtained, the chances of it being derived from the oxidation of ethanol are slight. The low boiling point of acetaldehyde and the negative Fehling's test of the fraction obviate its presence in the original fractions.

The hydrazone (m.p. 147° C.) from the pot residue precipitate is evidently derived from a three-carbon mono-carbonyl compound containing a non-carbonyl oxygen atom. This is interesting in light of Powick's (8) postulation that epihydrin aldehyde is present in oxidized fats. Theoretical considerations seem to eliminate epihydrin aldehyde as the precursor of this particular derivative. The reported absorption maximum of acrolein—2,4-dinitrophenylhydrazone is 366 mμ (1). Heilbron *et al.* (2) have reported that the epoxide group has light absorbing properties similar to those of carbon-to-carbon double bonds. This would suggest that the absorption maximum of epihydrin aldehyde—2,4-dinitrophenylhydrazone should be very close to 366 mμ. Such is not the case with this derivative, the absorption maximum being at 356–357 mμ. The reported melting point of acetol—2,4-dinitrophenylhydrazone is 127.5 to 129.5° C. (11), which would eliminate acetol as the parent compound. Attempts to convert the hydrazone to an osazone gave questionable results. When the hydrazone was gently refluxed with an excess of 2,4-dinitrophenylhydrazine in 2 *N* HCl, a small amount of dark brown precipitate formed in the hot reaction mixture after about 30 min. of refluxing. This precipitate gave a purple color with alcoholic NaOH, typical of 2,4-dinitrophenylosazones (7, 11). This suggests that the parent compound may have been lactaldehyde. Some serious doubts were cast upon this conjecture, however, when a subsequent control experiment showed that the

known 2,4-dinitrophenylhydrazone of butanal gave the same kind of precipitate when refluxed under the same conditions. Attempts to form an osazone under less strenuous conditions were unsuccessful. One would expect that lactaldehyde would form an osazone under these less strenuous conditions. The above observations and considerations would make 2-hydroxypropanal the best tentative structure that can be assigned to the parent compound with the available data. This discussion has not mentioned the possibility that the hydrazone may have resulted from decomposition of the fraction during the reaction. This is a distinct possibility, especially since a similar explanation was used to explain the acetaldehyde derivative from the previously reported fraction (2, 3, 4 combined).

The hydrazone (m.p. 115 to 116° C.) from the pot residue filtrate was derived from a carbonyl compound having the formula $C_{12}H_{20-22}O$. The absorption maximum of the hydrazone at 377 to 378 mμ suggests a carbon-to-carbon double bond conjugated with the carbonyl group. Whether the parent compound was an aldehyde or a ketone is not known, but the appearance of this 12-carbon unsaturated compound in the pot residue would suggest that the fractional distillation of the neutral fraction as carried out did not result in any appreciable decomposition of the compounds in the fraction.

The spectrophotometric evidence on the 2,4-dinitrophenylhydrazones obtained in this investigation was interpreted in the light of a study made by Braude and Jones (1). They have shown that the hydrazones of carbonyl compounds having a double bond conjugated with the carbonyl group exhibit maximum light absorption in the region 370 to 380 mμ, whereas most hydrazones of straight-chain saturated carbonyl compounds absorb at 356 to 360 mμ.

It is recognized that the conditions of autoxidation may have a profound influence on the types of degradation compounds formed in fats. The type of oxidation used in this study may have been specifically conducive to the formation of α, β-unsaturated carbonyl compounds. It is noteworthy that the volatile neutral fractions obtained from the oxidized milk fat simulated the typical odor of oxidized fat. It is interesting that no derivatives of heptanal, nonanal or similar saturated aldehydes were obtained. This is contrary to expectations in the light of previous reports (5, 9).

Due to the inherent complexity of the autoxidative process in unsaturated fats, any proposed structures for the derivatives isolated in this study would be, at best, purely speculative. Postulation that the carbonyl compounds were straight chained and not branched or cyclic may not be well founded due to the possibility of polymerization and condensation reactions taking place during the oxidation process.

Proof of the exact structure of the derivatives will require more exacting chemical study as well as the synthesis of authentic compounds and comparison of their properties with the properties of the compounds from the fat.

Synthetic α, β-unsaturated ketones. It has been reported that α, β-unsaturated ketones may be synthesized by a Friedel-Crafts reaction of an olefin with an acid chloride (4, 6). McMahon *et al.* (6) have used the reaction to prepare 1-pentene-3-one from ethylene and propionyl chloride. In order to gain a com-

parison of the properties of authentic compounds with those isolated in this study, some preliminary work has been done on the synthesis of α, β-unsaturated ketones. The method used was essentially that of McMahon *et al.* (6). A crude yield of 1-octene-3-one was obtained from the reaction of ethylene with caproyl chloride and crude 3-decene-5-one was prepared from 1-butene and caproyl chloride. The products have not been purified to an extent sufficient to make adequate organoleptic evaluation; however, neither one of the crude ketones seemed to have odors similar to the volatile material from oxidized fat. The reaction of the crude, 1-octene-3-one with 2,4-dinitrophenylhydrazine yielded two distinct derivatives, amber plates from ethanol melting at 121° C. and a red oil insoluble in hot ethanol. The crude 3-decene-5-one when reacted with 2,4-dinitrophenylhydrazine yielded red needles from ethanol melting at 107° C.

The tentative indications from this preliminary work are that the ethanol-insoluble red oils occasionally encountered when 2,4-dinitrophenylhydrazine is reacted with volatile oxidized fat material may be derived from 1-ene-3-one types of ketones. It also appears that none of the crystalline derivatives obtained in this study from oxidized fat were derived from the 1-ene-3-one type of ketone.

CONCLUSIONS

The preparation of 2,4-dinitrophenylhydrazones from volatile neutral material from oxidized milk fat indicated parent carbonyl compounds of the following emperical formulae: $C_3H_{4\text{-}6}O_2$, $C_7H_{12}O$, $C_9H_{16}O$, $C_{12}H_{20\text{-}22}O$. Theoretical considerations have indicated that the first compound was 2-hydroxypropanal. The C_7 and C_9 compounds were unsaturated ketones with the former containing an isolated ethylenic bond, while the latter was an α, β-unsaturated ketone. Neither of the ketones was methyl ketone. The C_{12} compound was an α, β-unsaturated carbonyl compound with no evidence being obtained to indicate whether it was an aldehyde or ketone.

ACKNOWLEDGMENTS

The authors are indebted to the Microchemical Department of Hoffman-LaRoche, Inc., Nutley, N. J., under the direction of A. Steyermark, for the performance of elemental microanalyses in connection with this research.

The interest and advice of Stuart Patton and E. F. Price of this department are gratefully acknowledged.

REFERENCES

(1) Braude, E. A., and Jones, E. R. H. Studies in Light Absorption. Part II. 2,4-dinitrophenylhydrazones. J. Chem. Soc., 498–503. 1945

(2) Heilbron, I M., Johnson, A. W., Jones, E. R. H., and Spinks, A. Studies in the Poylene Series. Part V. Synthesis of Vitamin A and Analogues. J. Chem. Soc., 727–733. 1942.

(3) Keeney, M., and Doan, F. J. Studies on Oxidized Milk Fat. I. Observations on the Chemical Properties of the Volatile Flavor Material from Oxidized Milk Fat. J. Dairy Sci., **34**: 713–718. 1951.

(4) Krapivin, S. Action of Acetyl Halides on Unsaturated Hydrocarbons in the Presence of Aluminum Halides. Bull. Soc. Imp. Nat. Moscow, 1–176, 1908. (Cited in Chem. Abs. **5**: 1281. 1911.)

(5) LEA, C. H. *Rancidity in Edible Fats.* Chemical Publishing Co., New York, N. Y. 1939.

(6) MCMAHON, E. M., ROPER, J. N., UTERMOHLEN, W. P., HASEK, R. H., HARRIS, R. C., AND BRANT, J. H. Preparation and Properties of Ethyl Vinyl Ketone and Methyl Isopropenyl Ketone J. Am. Chem. Soc., **70**: 2971–2977. 1948.

(7) NEUBERG, C., AND STRAUSS, E. Microdetermination of Dicarbonyl Compounds on the Coloring of Nitro Compounds in Alkaline Solutions. Arch. Biochem., **7**: 211–230. 1945.

(8) POWICK, W. C. Compounds Developed in Rancid Fats, with Observations on the Mechanism of their Formation. J. Agr. Research, **26**: 323–362. 1923.

(9) SCALA, A. Su di alcune sostauze volatili e non volatili che si formano nell'irrancidimento dei grassi. Gaz. chim. Ital., **38i**: 307–327. 1908.

(10) SHRINER, R. L., AND FUSON, R. C. *The Systematic Identification of Organic Compounds.* pp. 138–140. John Wiley & Sons, Inc., New York. 1948.

(11) STRAIN, H. H. 3-Nitrobenzohydrazones, 2,4-Dinitrophenylhydrazones and the Separation of Hydrazones by Adsorption. J. Am. Chem. Soc., **57**: 758–761. 1935.

STUDIES ON OXIDIZED MILK FAT. III. CHEMICAL AND ORGANOLEPTIC PROPERTIES OF VOLATILE MATERIAL OBTAINED BY FRACTIONATION WITH VARIOUS SOLVENTS AND GIRARD'S REAGENT[1]

MARK KEENEY[2] AND F. J. DOAN

Department of Dairy Husbandry, The Pennsylvania Agricultural Experiment Station, State College

It has been reported previously that the volatile neutral material from the vacuum distillation of oxidized milk fat contained carbonyl compounds, that some of these compounds appeared to be unsaturated ketones and that the carbonyl compounds were mainly responsible for the characteristic odor of the oxidized fat (4, 5). The present study involved the isolation of various classes of compounds from the distillate of oxidized milk fat and the correlation of chemical properties with organoleptic properties.

EXPERIMENTAL

The batch of liquid milk fat described in the previous paper (5) was allowed to stand in the laboratory exposed to diffuse sunlight for 7 mo., after which it was vacuum-distilled in the previously described manner (4), except that the oven temperature was maintained between 140 to 150° C. instead of between 160 and 180° C. Two and one-half kg. of fat yielded 10 g. of volatile material. Figure 1 illustrates how the volatile material was separated into various fractions.

Use of Girard's reagent. The volatile neutral fraction (fraction I, figure 1) was further fractionated by a modification of the method of Girard and Sandulesco (2). This method originally was devised for the isolation of keto-steroids and has found excellent acceptance in other fields requiring the isolation of pure ketones. Girard's "T" reagent is trimethylammoniumacetylhydrazine hydrochloride, and, under the proper reaction conditions, it forms water soluble hydrazones with a wide variety of carbonyl compounds. The usual procedure is to react the dry test material with an excess of Girard's reagent in absolute alcohol (ethyl or methyl) containing 10 per cent by volume of glacial acetic acid. After the reaction is completed, the mixture is poured into ice water containing enough base to neutralize nine-tenths of the acetic acid. The stable water solutions of the hydrazones are extracted with ether to remove non-carbonyl substances. They are made 1*N* with mineral acid which selectively hydrolyzes the keto-hydrazones and the regenerated ketones then are extracted with ether. Aldehyde-hydrazones are difficult to hydrolyze and the method is not feasible for the isolation of pure aldehydes.

The method as employed in this experiment involved the use of 10 per cent by

Received for publication Feb. 25, 1951.

[1] Authorized for publication on Feb. 16, 1951, as paper no. 1656 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Present address: Dairy Department, University of Maryland, College Park.

weight of acetic acid (instead of by volume) and less ice water than usually is recommended. Even with these slight modifications, the method as employed seemed to give satisfactory results. The previous experiments (4, 5) had indicated the presence of unsaturated ketones in the volatile neutral fraction from

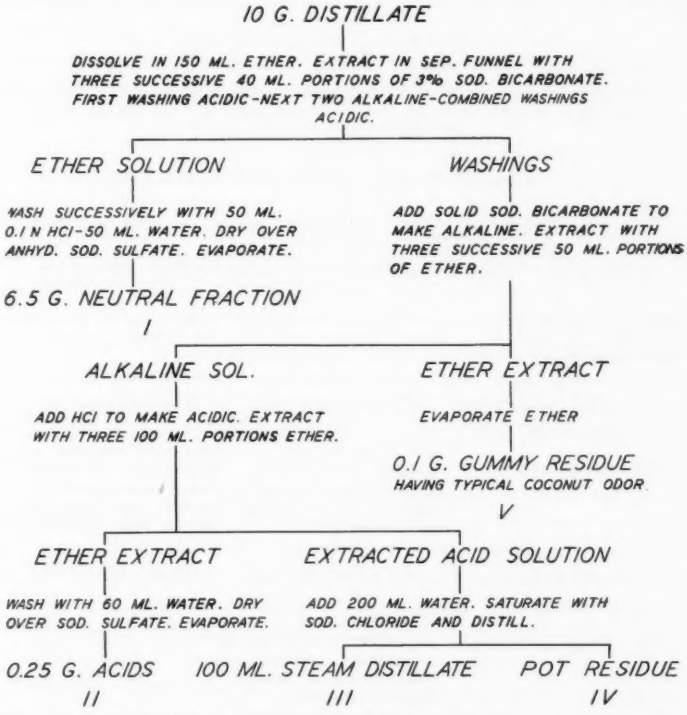

10 G. DISTILLATE
DISSOLVE IN 150 ML. ETHER. EXTRACT IN SEP. FUNNEL WITH THREE SUCCESSIVE 40 ML. PORTIONS OF 3% SOD. BICARBONATE. FIRST WASHING ACIDIC-NEXT TWO ALKALINE-COMBINED WASHINGS ACIDIC.
ETHER SOLUTION
WASHINGS
WASH SUCCESSIVELY WITH 50 ML. 0.1 N HCl-50 ML. WATER. DRY OVER ANHYD. SOD. SULFATE. EVAPORATE.
ADD SOLID SOD. BICARBONATE TO MAKE ALKALINE. EXTRACT WITH THREE SUCCESSIVE 50 ML. PORTIONS OF ETHER.
6.5 G. NEUTRAL FRACTION
I
ALKALINE SOL.
ETHER EXTRACT
ADD HCl TO MAKE ACIDIC. EXTRACT WITH THREE 100 ML. PORTIONS ETHER.
EVAPORATE ETHER
0.1 G. GUMMY RESIDUE
HAVING TYPICAL COCONUT ODOR
V
ETHER EXTRACT
EXTRACTED ACID SOLUTION
WASH WITH 60 ML. WATER. DRY OVER SOD. SULFATE. EVAPORATE.
ADD 200 ML. WATER. SATURATE WITH SOD. CHLORIDE AND DISTILL.
0.25 G. ACIDS
II
100 ML. STEAM DISTILLATE
III
POT RESIDUE
IV

FIG. 1. Solvent fractionation of volatile material from oxidized milk fat.

oxidized milk fat, so the Girard procedure was used with this lot of fat in an attempt at purification of the ketones. The procedure is outlined in figure 2.

Non-carbonyl neutral fraction. This fraction (A, figure 2) exhibited some properties which were not consistent with its label. In order to test the efficacy of the Girard procedure, the freshly isolated fraction was tested for carbonyl compounds with 2,4-dinitrophenylhydrazine reagent and was found to react negatively. The fraction had a mild ethereal odor with a slight suggestion of fruitiness and the refractive index was n_D^{25} 1.4350. When the fraction was titrated in neutral ethanol with KOH, a neutralization equivalent of 380 was obtained. This was determined by dissolving 119 mg. of fraction A (figure 2) in 8 ml. of neutral ethanol and titrating with 0.3462 *N* KOH to the phenolphthalein end point. The material seemed to titrate as a true acid and consumed 0.90 ml. of base. The original slight fruity odor was dissipated by alkali and the odor of the neutralized reaction mixture was oily with a slight suggestion of

fishiness. The fishiness may have resulted from amine degradation compounds from Girard's reagent. Acidification of some of the neutral reaction mixture resulted in a reappearance of the slight fruity odor of the fraction. Addition of 2,4-dinitrophenylhydrazine and H_2SO_4 to the neutral ethanol solution resulted in no ready precipitate. However, after standing overnight, a small

6.0 G. NEUTRAL FRACTION
ADD TO 100 ML. ABSOLUTE METHANOL CONTAINING 8.77 G. GLACIAL ACETIC. THEN ADD 12 G. GIRARDS T REAGENT. REFLUX 1.5 HR. COOL AND POUR INTO 800 ML. ICE WATER CONTAINING 7.07 G. SOD. CARBONATE.
AQUEOUS SOLUTION OF HYDRAZONES
EXTRACT WITH THREE SUCCESSIVE 200 ML. PORTIONS OF ETHER.
ETHER EXTRACT
EXTRACTED HYDRAZONE SOLUTION
WASH WITH 100 ML. WATER. DRY OVER SOD. SULFATE. EVAPORATE ETHER.
ADD 33 ML. CONC. HCl LET STAND ONE HR. AT ROOM TEMP. EXTRACT WITH TWO SUCCESSIVE 200 ML. PORTIONS OF ETHER.
1.2 G. NON-CARBONYL NEUTRAL FRACTION
A
EXTRACTED SOL
ETHER EXTRACT
LET STAND ONE HR. EXTRACT WITH TWO 200 ML. PORTIONS OF ETHER. WASH AND DRY AS BEFORE. EVAPORATE ETHER.
WASH WITH 2% SOD. BICARBONATE AND WATER UNTIL WASHINGS ARE NEUTRAL. DRY ETHER OVER SOD. SULFATE. EVAPORATE.
0.17 G. KETONES
C
EXTRACTED SOL.
0.70 G. KETONES
B
ADD 102 ML. CONC. HCl. LET STAND TWO HR. EXTRACT WITH TWO 200 ML. PORTIONS ETHER. WASH AND DRY AS BEFORE. EVAPORATE ETHER.
EXTRACTED SOLUTION
0.08 G. KETONES
D

FIG. 2. Separation of volatile neutral fraction from oxidized milk fat with Girard's "T" reagent.

amount of red oil precipitate had formed and the reaction mixture had assumed an extreme sharp fruity odor.

Ether-soluble acids. It was reported in preliminary work on solvent fractionation of the volatile material from oxidized milk fat that the use of Na_2CO_3 or K_2CO_3 for the extraction of acids resulted in an ether-soluble acid fraction which soon developed a typical coconut odor (3). In some cases, this odor development took place over night at 4° C., while in other cases, a few days at room temperature were required for coconut odor development. The ether-soluble acid fraction (II, figure 1) from the present batch of fat, in which $NaHCO_3$ was used for acid extraction, did not develop a coconut odor when stored for several

weeks at either room temperature or 4° C. It will be noticed that fraction V (figure 1) had a typical coconut odor.

One-tenth g. of fraction II (figure 1) was reacted with 0.2 g. of 2,4-dinitrophenylhydrazine in 20 ml. of 2 *N* HCl on a steam bath. Some dark brown precipitate formed immediately and after 5 min. on the steam bath, the reaction mixture was set aside to stand over night at room temperature. This resulted in the formation of about 150 mg. of long amber-colored needles. Recrystallization of these needles from chloroform gave small yellow-orange rhomboid crystals (m.p. 195 to 196° C.). Elemental analysis indicated the crystals to be derived from acetic acid. The reaction of acetic acid with 2,4-dinitrophenylhydrazine in 2 *N* HCl over night at room temperature gave yellow-orange crystals (m.p. 195 to 196° C.). A mixed melting point of these crystals with the above derivative showed no depression. The odor of the ether-soluble acid fraction suggested the presence of six- or eight-carbon acids; however, no compounds other than acetic acid were identified in this fraction. The dark brown precipitate which formed early in the reaction could not be purified to a sharp melting point.

Ketone fraction. The main ketone fraction (B, figure 2) had an odor which was similar to the ketone-containing fractions reported in the previous paper (5). This fraction seemed to react quantitatively and readily with 2,4-dinitrophenylhydrazine. Preliminary work with the ketone fraction has resulted in the easy preparation of three different crude 2,4-dinitrophenylhydrazones: red needles (m.p. 108–110° C.), yellow orange crystals (m.p. 65–70° C.) and a dark red alcohol-insoluble low-melting hydrazone. As yet, these derivatives have not been submitted to elemental analysis. The ketone fraction exhibited a negative iodoform test, negative Fehlings' test and the refractive index was n_D^{25} 1.4375. Fractions C and D (figure 2) had odors similar to the main ketone fraction.

Water- and bicarbonate-soluble fractions. It is evident from the data in figure 1 that the volatile material from this lot of liquid fat contained an appreciable quantity of water-soluble acids (more than enough to neutralize 3.6 g. $NaHCO_3$). In comparison to the water-soluble acids, the quantity of ether-soluble acids was small. This would suggest one or both of two possibilities. Either the inherent mechanism and extent of oxidation resulted in a relatively large amount of water-soluble acids or the method of extraction ($NaHCO_3$) did not remove all of the potential ether-soluble acids.

Fractions III and IV (figure 1) were obtained by distilling the saturated NaCl solution in a 500-ml. distilling flask at atmospheric pressure until 100 ml. of distillate was collected. By the end of the distillation period, the distilling flask, containing fraction IV, had some insoluble dark resin formed in it. A polymerizing and browning type of reaction was evidently being catalyzed by the distillation. Both fractions III and IV reacted with 2,4-dinitrophenylhydrazine to form precipitates, but no satisfactory derivative could be obtained from either fraction. The reaction of fraction III and various other fractions from the oxidized fat in the Kreis test and $FeCl_3$ test has been reported elsewhere (7).

Flavor imparted to milk by certain fractions. A comparison was made of the flavor imparted to milk by the so-called non-carbonyl neutral fraction (A,

figure 2) and the ketone fraction (B, figure 2). Much more of fraction A was necessary to impart a detectable off-flavor than was needed when fraction B or the whole neutral fraction were added to milk. One-half ppm. of fraction A could not be detected consistently, while 5 ppm. were readily detectable. Only 0.05 ppm. of fraction B were required to impart a distinct off-flavor to milk. While the ketone fraction imparted an off flavor that was suggestive of oxidized milk, the character of the flavor seemed to be distinctly different than that normally associated with oxidized milk. The non-carbonyl neutral fraction, when added to milk, closely simulated the flavor of oxidized milk; however, the most faithful representation of oxidized milk resulted when 0.5 ppm. of fraction A plus 0.05 ppm. of fraction B were added to milk.

Control experiments. While the milk fat used in this study was being oxidized, its control counterpart was being held in cold storage (5° C.). Distillation of the control fat by the same method used on the oxidized fat resulted in a negligible amount of distillate (one drop from 400 g. of fat) which did not have the characteristic odor of oxidized fat.

In order to obtain some information on the effect of longer distillation time on the kind of volatile material from oxidized milk fat, 400 g. of the liquid fat, which already had been distilled as described, was vacuum distilled again for 45 min. with an oven temperature 140 to 150° C. The yield of volatile material amounted to about one-third of that from the first distillation and the odor was similar. The only further observation made on this material was to react it with 2,4-dinitrophenylhydrazine. Chromatography of the hydrazones on a 2:1 alumina-bentonite column (2.5 × 20 cm.) from petroleum ether, developed and eluted with gradually increasing quantities of ethyl ether in petroleum ether to 100 per cent ethyl ether, resulted in a major band which yielded 40 mg. red-orange needles (m.p. 109° C.), when recrystallized from ethanol. The details for the preparation and chromatography of the derivative are the same as previously described (5). The absorption maximum of the hydrazone in 95 per cent ethanol was at 378–379 mμ. Elemental analysis of the derivative gave 57.14 per cent carbon, 6.47 per cent hydrogen and 16.83 per cent nitrogen. The calculated analysis for the derivative of a parent compound of the formula $C_{10}H_{18}O$ is 57.47 per cent carbon, 6.63 per cent hydrogen and 16.76 per cent nitrogen. The wave length of maximum light absorption indicated that the derivative was from an α, β-unsaturated carbonyl compound (1). No direct evidence was obtained to show whether it was derived from an aldehyde or ketone.

DISCUSSION

It was recognized previously (4) that the oxidized milk fat being used in these studies was subjected to more extreme oxidizing conditions than would normally be encountered in dairy products and that the relatively high distillation temperatures probably caused further deterioration of the fat. Although much research has been done and many theories have been advanced relative to the mechanism of fat oxidation, an actual understanding of the over-all process is still a matter of conjecture. It is well known that the odor and flavor of fats

and fat-containing products change during various periods of the oxidation process. The question of whether or not this change in organoleptic character is due to an orderly and definite chemical process is worthy of consideration. One answer may be that a group of susceptible compounds is oxidized to a distinctly different group of compounds, having flavor characteristics, in an orderly chain-like process. Another answer may be that there are different paths for the oxidation process to follow after initiation, depending on conditions, with each path leading to different types of compounds and consequently variations in flavor.

The results of this study indicate that even in advanced stages of oxidation it is possible to isolate various flavor fractions which are typical of different stages in the oxidation process. It appears, therefore, that the change in odor and flavor during oxidation is due to a blending of different flavor compounds with the organoleptic character of certain of them being completely masked by others which are more potent.

The observations on the coconut and fruity flavors of various fractions are of practical interest and significance. Whole milk powder when freshly made or early in storage often develops a distinct off-flavor which is variously described as typical of whole milk powder, like coconut or stale (6). While the development of this defect appears to be oxidative in nature, the flavor is distinctly different from that which develops later in storage and which generally is termed oxidized flavor. This early storage defect seems to be peculiar to and directly attributable to the oxidative deterioration of dry milk fat (6).

The data reported in this and a previous paper (3) indicate that the coconut odor material from oxidized milk fat is marginally soluble in both water and ethyl ether and soluble in bases stronger than $NaHCO_3$. It is regenerated from these bases with acid. It has been postulated that the coconut odor development may have been due to lactone formation (3). The fruity odor of the non-carbonyl neutral fraction (A, figure 2) also may have been due to the same phenomenon. It is apparent that the coconut odor material is co-present with other more potent odor compounds and is masked by them in the more advanced stages of milk fat oxidation.

The use of Girard's reagent has confirmed previous indications (5) on the significance of ketones as contributors to the characteristic odor of oxidized milk fat. These ketones are not methyl ketones and the majority of them are unsaturated.

The addition of various purified flavor fractions from oxidized milk fat to milk demonstrated that a blending of different flavor compounds is necessary to reproduce the flavor of oxidized milk as it is encountered on the market.

The extremely small quantities of volatile material necessary to impart off-flavors to milk help to account for the difficulty which has been encountered in explaining the chemistry and mechanism of deteriorated-fat flavors in dairy products. This is true because odor and flavor are perceptible in far smaller concentrations than can be detected chemically and secondly, because the organoleptic reactions, while very sensitive, are subject to human error in interpretation and chemical tests cannot be resorted to for verification.

Control experiments have shown that while the high temperature used for distillation caused concomitant degradation of the fat, a necessary precursor to the volatile flavor material was autoxidation of the fat before distillation.

The aldehydes were not recovered from the Girard procedure and further study will be necessary to ascertain their role in the organoleptic properties of oxidized milk fat.

SUMMARY

The predominant odor compounds from the oxidized milk fat used in this study were ketones. None of these ketones were methyl ketones and a large proportion of them were unsaturated.

Presumptive evidence has been presented which suggests that lactones are to be found in the volatile material and these have been correlated with the fruity and coconut odor of certain fractions of the distillate.

The flavor of oxidized milk was closely simulated when the ketone fraction and non-carbonyl neutral fraction were mixed in milk in the proper proportions.

It has been shown that in the advanced stages of milk fat oxidation it is possible to isolate flavor fractions which are typical of earlier stages in the oxidation and that the change in organoleptic character of milk fat during oxidation is due to a blending of different flavor compounds.

The prolonged distillation of oxidized milk fat resulted in the formation of an α, β-unsaturated carbonyl compound of the formula $C_{10}H_{18}O$ and the properties of its 2,4-dinitrophenylhydrazone have been presented.

ACKNOWLEDGMENT

The authors are indebted to the Microchemical Division of Hoffmann-La Roche, Inc., Nutley, N. J., under the direction of A. Steyermark for the performance of elemental microanalyses in connection with this research.

REFERENCES

(1) Braude, E. A., and Jones, E. R. H. Studies in Light Absorption. Part II. 2,4-Dinitrophenylhydrazones. J. Chem. Soc., 498–503. 1945.

(2) Girard, A., and Sandulesco, G. Sur une nouvelle series de reactifs du groupe carbonyle, leur utilization a l'extraction des substances citeniques et a la caracterisation microchimique des aldehydes et cetones. Helv. Chim. Acta, **19**: 1095–1107. 1936.

(3) Keeney, M., and Doan, F. J. Studies on Oxidized Milk Fat. J. Dairy Sci., **33**: 397–398. 1950.

(4) Keeney, M., and Doan, F. J. Studies on Oxidized Milk Fat. I. Observations on the Chemical Properties of the Volatile Flavor Material from Oxidized Milk Fat. J. Dairy Sci., **34**: 713–718. 1951.

(5) Keeney, M., and Doan, F. J. Studies on Oxidized Milk Fat. II. Preparation of 2,4-Dinitrophenylhydrazones from the Volatile Material from Oxidized Milk Fat. J. Dairy Sci., **34**: 719–727. 1951.

(6) Mussett, A. T., Patton, S., and Dahle, C. D. Flavor Deterioration Associated with the Lipid Phase of Whole Milk Powder. J. Dairy Sci., **33**: 299–305. 1950.

(7) Patton, S., Keeney, M., and Kurtz, G. W. Some Observations on Compounds Producing the Kreis Color Reaction with Particular Reference to Compounds from Oxidized Milk Fat. J. Am. Oil Chem. Soc. In press.

THE EFFECT OF VITAMIN D ON THE UTILIZATION OF ENERGY AND PROTEIN OF THE RATION OF CALVES[1]

N. F. COLOVOS, H. A. KEENER, A. E. TEERI AND H. A. DAVIS

New Hampshire Agricultural Experiment Station, Durham

There is considerable evidence in the literature to show the importance of vitamin D in the nutrition of the calf (1, 2, 3, 8, 10, 17, 19). Much of the earlier work was concerned with the pathology of the calf on low vitamin D diets. Many of these experiments showed that neither Ca nor P can be utilized most efficiently without adequate vitamin D and that rickets, formerly a serious problem in calf raising, can be caused by a lack of this vitamin.

Previous research at this station (13) showed that vitamin A deficiency in the ration of the calf lowered the digestibility and the retention of the protein and the energy of the ration. The second phase of this physiological study, which is reported here, was undertaken to determine the extent to which vitamin D influences the digestion of the various feed constituents, the utilization of energy and nitrogen, the basal metabolic rate, and the chemical composition of the blood.

EXPERIMENTAL

The first part of this work was carried out during the winter and spring of 1945, the second part during the spring and summer of 1949. The experimental animals used were male Holstein calves. The first year there were two groups of three animals, each group consisting of one positive control and two deficient calves. The second year there were four pairs with each consisting of one positive control and one deficient animal. The members of each pair or group were as uniform in weight as possible and the allotment to the control or deficient ration was made on the basis of chance.

The calves were placed on the experiment a few days after birth and were kept in individual pens in a darkened room throughout the experiment. For the first 5 wk. the calves were given whole milk, the amount of which was gradually decreased as they were able to obtain their nutrient requirements from a dry ration consisting of two-thirds concentrates and one-third dried beet pulp. The concentrate part of the ration was a low vitamin D mixture composed of 100 lb. ground yellow corn, 100 lb. ground oats, 100 lb. wheat bran, 50 lb. soybean oil meal, 40 lb. non-fat dry milk solids, 4 lb. salt, and 4 lb. pulverized limestone. No hay was fed during the experiment.

After the feeding of whole milk was discontinued, each calf was given a carotene concentrate in amounts which would furnish 100 U.S.P. units of vitamin A equivalent per pound of body weight per day. In addition, the control calves were given irradiated brewers' yeast[2] so as to furnish vitamin D at a level of

Received for publication, Feb. 10, 1951.

[1] Scientific contribution no. 139 of the New Hampshire Agricultural Experiment Station.

[2] The yeast used during this experiment was supplied through the courtesy of Standard Brands Inc., New York.

10 I.U. per pound of body weight per day during the first experiment and at a level of 20 I.U. during the second. During the second experiment, the deficient animals were fed an equivalent amount of non-irradiated brewers' yeast in order that the deficient animals would receive the same amount of nitrogen from yeast as the control calves. The feeding of both the yeast and carotene was discontinued at the beginning of the collection period.

Periodic blood analyses were made to determine the Ca, inorganic P and alkaline phosphatase levels of the blood. During the second experiment, periodic basal metabolism measurements also were made 24 to 30 hr. after feeding and with the animals in a lying position.

When the vitamin D-deficient animals showed definite rachitic symptoms, such as arched back, big knees and sore joints, both the control and deficient animals of the pair or group were placed in calf-metabolism stalls constructed according to the plans of Loosli (12). These stalls permitted a separate collection of urine and feces. The digestion experiments, which were approximately 14 days in length, were conducted according to the routine procedures of this laboratory, as given by Colovos *et al.* (6). During the second experiment, the feed and feces also were analyzed for ether extract, crude fiber, ash and nitrogen-free extract, and the digestibility of these constituents was calculated. At the end of the collection period each animal was placed in the open circuit-type respiration chamber for three consecutive 12-hr. metabolism measurements. During these measurements, the animals were fed in the customary way and allowed normal activity. The metabolism measurements were carried out with the equipment and according to the methods described by Benedict *et al.* (4) and Colovos *et al.* (6). The calves were transported between the barn and the metabolism laboratory during darkness or else covered to prevent exposure to sunlight.

RESULTS AND DISCUSSION

Average values for the chemical composition of the concentrate mixture and of the beet pulp are given in table 1. There were slight differences in the composition of both the concentrate mixture and the beet pulp between mixtures or lots, so actual values were used in calculating the digestion and utilization data.

TABLE 1
Average composition of feeds

Feeds	Moisture	Protein	Ether extract	Fiber	N-free extract	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
Concentrate mixture	11.55	18.63	3.14	5.48	55.06	6.14
Beet pulp	12.31	8.54	0.35	18.46	54.44	5.90

The effect of vitamin D on the digestion of the various feed constituents is given in table 2. The average digestion coefficients for dry matter, protein and energy in the 1945 experiment were higher than those of the 1949 experiment. One explanation for this difference may be that there were differences in the quality of the ingredients used in the rations. It also may be attributed to a dif-

TABLE 2

The effect of vitamin D on digestion coefficients (corrected)[a]

Control calf no.	D.M.	Protein	Ether extract	Fiber	N.F.E.	Ash	Energy	Deficient calf no.	D.M.	Protein	Ether extract	Fiber	N-free extract	Ash	Energy
	(%)	(%)	(%)	(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)	(%)	(%)	(%)
1945															
48	79.3	79.8	.....	.....	.....	.....	78.0	46	76.6	72.8	.....	.....	.....	.....	76.3
								47	81.2	74.3	.....	.....	.....	.....	80.6
50	76.8	77.3	.....	.....	.....	.....	75.2	49	82.2	77.6	.....	.....	.....	.....	78.8
								51	81.3	78.8	.....	.....	.....	.....	80.2
Av.	78.2	78.6	.....	.....	.....	.....	76.6	Av.	80.3	75.8	.....	.....	.....	.....	78.4
1949															
86	77.6	69.6	59.9	62.8	85.6	55.4	75.1	87	76.6	66.6	55.1	59.0	86.3	39.2	76.3
90	77.9	76.4	81.3	43.4	85.2	58.6	77.4	88	79.1	75.0	79.8	59.8	87.0	47.5	78.8
93	78.2	75.3	60.7	59.2	84.6	61.0	76.9	89	78.9	74.7	65.2	60.0	87.3	40.8	78.0
92	73.6	75.4	81.8	28.2	85.3	57.7	74.6	91	72.8	68.2	78.7	36.9	83.1	49.8	72.4
Av.	76.8	74.2	70.9	48.4	85.2	58.2	76.0	Av.	76.9	71.1	67.7	53.9	85.9	44.3	76.4

[a] Because certain deficient animals sorted their mixture to some extent and consumed proportionately less of the more highly digestible concentrate mixture and more of less digestible beet pulp, it was necessary to correct the digestion coefficients in order that the effect of vitamin D deficiency could be ascertained. The values in this table have been corrected using calculated values from Morrison and allowing for the maximum effect from such sorting by assuming refuse was all grain.

TABLE 3

Effect of vitamin D on the utilization of energy and protein

Control calf no.	Energy			Protein			Deficient calf no.	Energy			Protein		
	Absorbed	Balance	Metaboliz-ability	Absorbed	Balance	Retention of absorbed		Absorbed	Balance	Metaboliz-ability	Absorbed	Balance	Retention of absorbed
	(*Cal.*)	(*Cal.*)	(%)	(*g.*)	(*g.*)	(%)		(*Cal.*)	(*Cal.*)	(%)	(*g.*)	(*g.*)	(%)
1945													
48	6,760	−161	67.8	275.35	121.94	44.3	46	6,610	−2,191	66.0	251.37	114.29	45.5
							47	5,597	.....	.....	150.34	38.99	25.9
50	6,466	+3	63.3	263.86	140.21	53.1	49	5,607	−360	70.1	218.41	97.87	44.8
							51	5,123	−63	70.7	199.69	106.77	53.4
Av.	6,613	−79	65.6	269.67	131.08	48.7	Av.	5,734	−571	68.9	204.95	89.5	42.4
1949													
86	7,158	−557	66.1	273.53	117.95	43.1	87	6,386	47	67.6	212.78	77.02	36.2
90	7,178	132	71.2	291.64	114.21	39.2	88	6,631	−724	73.2	238.20	79.14	33.2
92	6,570	−575	67.3	270.06	126.90	47.0	91	6,502	−630	64.2	236.59	102.79	43.4
93	8,059	1,631	71.4	319.58	175.94	55.1	89	6,743	−538	71.4	256.75	92.61	36.1
Av.	7,241	316	69.0	288.70	133.9	46.1	Av.	6,565	−461	69.1	236.08	87.9	37.2

ference in the age and size of the experimental animals. The digestion and utilization measurements in 1945 were made in a more advanced stage of vitamin D deficiency after the intake of the feed had decreased because of anorexia in the deficient animals. This may account for the erratic results obtained. To avoid this in 1949, the digestion balances were started as soon as slight symptoms of vitamin D-deficiency were observed. It should be noted, however, that vitamin D-deficiency symptoms were observed at a considerably earlier age in 1945.

The percentage digestibility of the protein of the ration was higher in the controls than in the deficient animals the second year. This difference was significant at the 5 per cent level. The average percentage digestibility of the energy $\left(\frac{\text{gross energy of feed-energy in feces}}{\text{gross energy of feed}} \times 100\right)$, however, was slightly higher in the deficient animals, although not significantly so. There were no differences in the digestibility of dry matter, ether extract or fiber. As might be expected, vitamin D deficiency lowered the digestibility of ash significantly, the difference being significant at the 1 per cent level.

TABLE 4

Effect of vitamin D in basal metabolism

Calf no.	Group	Calories per kilogram body wt. 24 hr. at ages of:				
		9 wk.	12 wk.	17 wk.	22 wk.	All ages Av.
86	Control	27.7	26.3	26.8	26.2	26.7
87	Deficient	28.2	27.1	31.6	29.7	29.1
90	Control	32.2	32.9	28.5	30.3	31.0
88	Deficient	33.5	35.5	36.9	31.6	34.4
92[a]	Control	.....	.....	.....	.....	.....
91	Deficient	.....	32.8	40.6	31.1	.....
93	Control	29.4	30.0	28.3	29.3	29.3
89	Deficient	31.9	37.7	29.6	32.5	32.9

[a] Calf no. 92 was off feed at beginning of experiment so comparable metabolic rate values are not available. This animal recovered, however, and a digestion balance was carried out at the end of the experiment.

The results of the utilization study as given in table 3 show that the calves were supplied more adequately with protein than with energy. A careful study of the data shows that both the protein and the energy of the ration were utilized more efficiently by the control animals. The difference in retention percentage of absorbed nitrogen between the control and deficient animals was significant at the 5 per cent level. This is clearly indicated by the balance studies shown in table 3. The higher metabolic rates and slower body gains of the deficient calves as shown in tables 4 and 5 are in agreement with the results of the balance studies. The apparent inconsistency between the gain in body weight during the experiment and the minus balance of energy during the metabolism measurement at the end of the experiment is due to the fact that the deficiency symptoms had become so acute in the deficient animals that most of them would not eat enough to meet requirements. When restricted to equivalent amounts of feed, some control animals also showed a minus balance of energy at this time.

The amount of protein absorbed was lower in relation to absorbed energy in

the deficient calves because of both a decrease in digestibility of protein and a lower protein consumption as the deficiency symptoms developed, a slight anorexia caused the calves to sort their feed, leaving proportionately more of the higher protein concentrate mixture than of the lower protein beet pulp. Thus, in addition to a favorable effect on protein digestion, vitamin D also increases the efficiency of protein utilization, either directly or through its effect on feed consumption and rate of growth. Support for the observation that vitamin D has a nutritional significance in addition to its relation to bone growth is furnished by Swanson and Clark (18).

It is not possible to arrive at any exact information on the utilization of the ash constituents because of the fact that relatively large amounts of mineral matter of metabolic origin may appear in the feces. One cannot say whether the increased excretion of mineral matter in the feces of the deficient calves was due to decreased absorption, to a greater elimination of mineral matter of metabolic origin or to both. However, the net effect of vitamin D on the mineral balance was very marked.

TABLE 5

Effect of vitamin D on body weight gains

Control calf no.	Time	Gain	Deficient calf no.	Time	Gain
	(*wk.*)	(*lb.*)		(*wk.*)	(*lb.*)
1945					
48	16	142.0	46	16	127.0
			47	16	124.0
50	13	97.0	49	13	93.0
			51	13	70.5
1949					
86	21	181.5	87	21	160.5
90	19	198.5	88	19	162.5
92[a]	19	177.5	91	19	191.0
93	21	192.5	89	21	159.5

[a] Off feed at beginning of experiment so gain is abnormally low.

The effect of vitamin D on the basal metabolism is shown in table 4. The first basal metabolism measurements were obtained about 1 mo. after the feeding of whole milk was discontinued. The control calves had a fairly constant rate of metabolism throughout the experiment. The deficient animals had a slightly higher metabolic rate at the time of the first measurements and it increased generally and sometimes markedly during the next two measurements. At the time of the final series of measurements, the basal metabolic rate of the deficient calves had decreased considerably but still was significantly higher than that of the controls. The reason for this drop is not known. It may represent the effect of some compensatory action on the part of the animal in adjusting itself to the deficiency. These results may be interpreted to mean that in addition to the effects of vitamin D deficiency already discussed there may be a further loss of nutrients due to an increase in the energy expenditures of the body.

The results of the blood studies as given in table 6 are averages of comparable values obtained after the appearance of deficiency symptoms. They are in agreement with the observation of Rupel *et al.* (17) and many others that vitamin

D deficiency in calves brings about a lowering of the Ca and inorganic P of the blood. Alkaline phosphatase activity, as determined by the method and expressed as Bodansky (5) units, was found to increase in the blood of the deficient calves. The differences in blood alkaline phosphatase and calcium between the control and deficient animals are significant at the 1 per cent level, while that for phosphorus is significant at the 5 per cent level. This is in agreement with the results of Hibbs *et al.* (9).

The accumulated effect of a lowered feed consumption, a decrease in the digestion of protein, a less efficient nitrogen utilization, a lowered digestion of mineral matter and an increased basal metabolism should be a decrease in body weight gains. That this did happen is shown by table 5. It is seen that, in general, the body weight gains of the deficient calves were considerably lower than for the control.

TABLE 6

Effect of vitamin D on average composition of blood (per ml. serum)

Control calf no.	Ca	P	Alkaline phosphatase	Deficient calf no.	Ca	P	Alkaline phosphatase
	(*mg.*)	(*mg.*)	(*units*)		(*mg.*)	(*mg.*)	(*units*)
1945							
48	11.6	8.8	4.6	46	9.6	9.2	10.6
				47	10.6	8.1	16.3
50	11.9	9.7	4.9	49	8.4	6.0	10.4
				51	8.3	5.0	10.1
Av.	11.8	9.3	4.8	Av.	9.2	7.1	12.4
1949							
86	10.8	7.2	5.4	87	6.6	5.4	9.6
90	10.9	7.4	5.7	88	7.0	5.6	8.2
93	11.0	7.3	6.2	89	8.0	5.6	8.2
92	10.6	7.7	5.5	91	10.5	6.7	6.9
Av.	10.8	7.4	5.7	Av.	8.0	5.8	8.4

SUMMARY AND CONCLUSIONS

Fourteen Holstein bull calves were used in a study to determine the effect of vitamin D on the digestion and utilization of the protein and the energy of the ration; the metabolic coefficients for ether extract, nitrogen-free extract, fiber and ash also were determined. Periodic basal metabolism measurements were made on the same eight calves.

Both the digestion of the feed protein and retention of the absorbed nitrogen were lowered by vitamin D deficiency. The digestibility of ash likewise was affected adversely by a lack of vitamin D, but the digestion of dry matter, ether-extract, fiber, nitrogen-free extract and energy were not affected significantly. The efficiency of energy and protein utilization was significantly decreased. The basal metabolic rate was increased in the calves as a result of vitamin D deficiency. Blood Ca and inorganic P were lowered by the deficiency, while the alkaline phosphatase activity was increased. The deficiency slowed gains in body weight and produced the usual symptoms of rickets, such as arched back, large knees and soreness of joints.

It was found that vitamin D is needed by calves not only to promote normal bone growth, but also to permit efficient digestion and utilization of certain feed nutrients and a normal metabolic rate.

REFERENCES

(1) Bechdel, S. I., Landsburg, K. G., and Hill, O. J. Rickets in Calves. Pa. Agr. Expt. Bull. 291. 1933.

(2) Bechdel, S. I., Hilston, N. W., Guerrant, N. B., and Dutcher, R. A. The Vitamin D Requirements of Dairy Calves. Pa. Agr. Expt. Sta. Bull. 364. 1938.

(3) Bechtel, H. E., Hallman, E. T., Huffman, C. F., and Duncan, C. W. Pathology of Rickets in Dairy Calves. Mich. Agr. Expt. Sta. Tech. Bull. 150. 1936.

(4) Benedict, F. G., Coropatchinsky, V., and Ritzman, E. G. Technik der Messung des Gesamtstoffwechsels und des Energiebedarfes von Haustieren. Abderhalden's Handb. d. biolog. Arbeitsmethoden, Abt. IV, Teil, **13**: 619. 1934.

(5) Bodansky, A. Phosphatase Studies Determination of Serum Phosphatase. J. Biol. Chem., **101**: 93–104. 1933.

(6) Colovos, N. F., Keener, H. A., Prescott, J. R., and Teeri, A. E. The Nutritive Value of Timothy Hay at Different Stages of Maturity as Compared with Second-cutting Clover Hay. J. Dairy Sci., **32**: 659–664. 1949.

(7) Duncan, C. W., and Huffman, C. F. Vitamin D. Studies in Cattle. III. Influence of Solar Ultraviolet Radiation upon the Blood Chemistry and Mineral Metabolism of Dairy Calves. J. Dairy Sci., **19**: 291–303. 1936.

(8) Hart, E. B., Steenbock, H., Kline, O. L., and Humphrey, G. C. The Influence of Irradiated Yeast on the Calcium and Phosphorus Metabolism. J. Biol. Chem., **45**: 343. 1930.

(9) Hibbs, J. W., Krauss, W. E., Monroe, C. F., and Pounden, W. D. A Report on the Occurrence of Rickets in Calves under Farm Conditions. Ohio Bimon. Bull., **30** (232): 3–8. 1945.

(10) Huffman, C. F., Duncan, C. W., and Lightfoot, C. C. Vitamin D Studies in Cattle. I. The Antirachitic Value of Hay in the Ration of Dairy Cattle. J. Dairy Sci., **18**: 511–526. 1935.

(11) Huffman, C. F., and Duncan, C. W. Vitamin Studies in Cattle. II. The Vitamin D-sparing Action of Magnesium in the Ration of Dairy Cattle. J. Dairy Sci., **18**: 605–620. 1935.

(12) Loosli, J. K. Cornell University. Special correspondence. Apr. 3, 1943.

(13) Morrison, F. B. *Feeds and Feeding.* 21st ed., Ithaca, N. Y.

(14) Maynard, L. A. *Animal Nutrition.* McGraw-Hill Co., New York and London, p. 193. 1947.

(15) Presnell, A. K. The Relation of Vitamin D to Skin Respiration. J. Biol. Chem., **121**: 5–8. 1937.

(16) Ritzman, E. G., Colovos, N. F., Keener, H. A., and Teeri, A. E. Influence of Vitamin A on the Utilization of Energy and Protein by Calves. N. H. Agr. Expt. Sta. Tech. Bull. 87. 1945.

(17) Rupel, I. W., Bohstedt, G., and Hart, E. G. Vitamin D in the Nutrition of the Dairy Calf. Wis. Agr. Expt. Sta. Research Bull. 115. 1933.

(18) Swanson, P. P., and Clark, H. E. Annual Rev. Biochem., **XIX**: 248. 1950.

(19) Wallis, G. C., Palmer, L. S., and Gullickson, T. W. The Relation of Vitamin D to Calcium and Phosphorus Retention in Cattle as Shown by Balance Trials. J. Dairy Sci., **18**: 213–228. 1935.

DETERMINATION OF HEAT-PRODUCED REDUCING SUBSTANCES IN MILK WITH PORPHYREXIDE

AUBREY P. STEWART, JR.
Research Laboratories of Golden State Co., Ltd., San Francisco, California

The importance of proper heat treatment in achieving desirable properties in certain milk products has been recognized more and more in recent years. Proper application of heat during processing has allowed the manufacturer of sweetened condensed milk and evaporated milk to control viscosity of his products. Proper heat treatment of skimmilk prior to drying has yielded nonfat dry milk solids of improved baking properties.

One very significant result of heating milk is the formation of certain reducing substances. These substances seem to be formed by the interreaction between the milk proteins and lactose (16). Their presence in milk products which have undergone heat treatment during processing is of considerable interest. It is believed that reducing substances in nonfat dry milk solids contribute in some way to the fermentation in bread doughs. Most certainly, heat-produced reducing substances serve as antioxidants and, thus, become important to the keeping properties of dry whole, frozen and canned milks. Reducing substances also are related directly to cooked flavor in milk products.

With the realization of the importance of heat-produced reducing substances, efforts have been made in recent years to find a method of accurate analysis. The nitroprusside test was one of the first to be used for this purpose (4, 8, 9). Subsequently, other methods were developed, including reaction of the reducing substances with 2,6-dichlorophenolindophenol (7, 16), thiamin disulfide (6), ferricyanide (1, 2) and o-iodosobenzoate (12). All of these methods have been used as important research tools in learning more about the function of reducing substances in milk. However, it is the general belief that no single method is completely satisfactory, and that new or improved methods would be of aid in further studies.

The titration of reducing substances with 2,6-dichlorophenolindophenol has been employed by many investigators, but the reaction is slow, the end-point difficult to read and the indophenol dye reacts so incompletely that the method is relatively insensitive.

The ferricyanide method can be used to yield only comparative results. The effects of factors such as pH, time and temperature used for the reaction with ferricyanide and sample size employed are so great that completely different results can be obtained by varying any one of the factors. Furthermore, it can be demonstrated that unheated milk will cause considerable reduction of ferricyanide, which makes the method less valuable as a measure of strictly heat-produced reducing substances. Reducing substances measured by the ferricyanide method do not correlate satisfactorily with cooked flavor.

In searching for a new method for the determination of heat-produced reduc-

Received for publication Feb. 12, 1951.

ing substances, the following requirements were considered: (a) The method should measure only heat-produced reducing substances; (b) the measurement should be sensitive and as nearly quantitative as possible; (c) the measurement should be made of the reducing substances existing at the normal pH of milk and room temperature; (d) the measurement should indicate actual amounts rather than comparative amounts of the reducing substances.

A review of the literature revealed that the dyes porphyrexide and porphyrindin are two of the most powerful oxidizing agents known in organic chemistry. Porphyrindin has been used to measure sulfhydryl groups present in proteins (5, 10). The O-R potential at pH 7.0 of porphyrexide is +0.725 volt and of porphyrindin, +0.565 volt, as compared with +0.217 volt for 2,6-dichlorophenol-indophenol.

Initial attempts to use porphyrindin and porphyrexide in the direct titration of reducing substances in milk were unsuccessful. Although the dyes underwent a color change on reduction, the color intensity was too low to achieve a satisfactory end-point. Also, it was found that at the normal pH of milk the dyes were absorbed on the milk protein to some extent, interfering with the direct titration. Further work finally yielded a porphyrexide method which has since proved valuable as a measure of reducing substances. It was discovered that by adding p-aminodimethylaniline sulfate to the milk, excess porphyrexide dye remaining after reaction with heat-produced reducing substances would yield a proportional amount of color sufficiently intense to measure accurately. Furthermore, this color could be passed quantitatively into a clear filtrate after precipitating the protein with H_2SO_4 and NaCl. The color then could be measured with a colorimeter.

DETERMINATION OF REDUCING SUBSTANCES

Reagents. (a) Porphyrexide dye solution: Porphyrexide dye may be synthesized according to the method of Piloty and Schwerin (14) and Kuhn and Franke (11), as modified by Lillevik *et al.* (13). The spirocyclohexyl porphyrexide analog is easier to synthesize, however, and gives equivalent results. The synthesis of this porphyrexide analog is described by Porter and Hellerman (15). A commercially obtained porphyrexide dye also has been used satisfactorily.[1]

Weigh out on an analytical balance and dissolve in 100 ml. water sufficient dye so that the resulting strength will be between 0.35 and 0.37 mg. per milliliter, calculated as ascorbic acid. In the case of porphyrexide, this will require approximately 60 mg. dye and for the analog approximately 95 mg., depending on the exact purity. The dye solution should be made up fresh each time a series of samples is to be analyzed.

(b) Metaphosphoric acid—12 per cent: Dissolve 12 g. C.P. metaphosphoric acid sticks or pellets in water and make up to total volume of 100 ml. Prepare fresh each week.

(c) Ferrous solution: Weigh exactly 5.000 g. C.P. $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ on an analytical balance and transfer to a 1000-ml. volumetric flask containing

[1] Bios Laboratories, Inc., 17 West 60th St., New York 23, N. Y.

about 300 ml. water and 10 ml. concentrated H_2SO_4. Dissolve and make up to 1,000 ml. total volume with water. Prepare fresh each week.

(d) Sulfuric acid—2.5 per cent: To 50 ml. water in a beaker, add slowly with stirring, 2.5 ml. C.P. concentrated H_2SO_4. Cool to room temperature, transfer to a 100-ml. volumetric flask and make up to volume with water.

(e) Indicator solution: Weigh exactly 0.100 g. p-aminodimethylaniline sulfate[2] on an analytical balance. Transfer to a test tube, add 10 ml. water and dissolve completely. Prepare fresh each day.

(f) Sodium chloride: Use reagent grade, small crystals.

Standardizing porphyrexide dye solution. The dye solution should be standardized either just before analyzing a series of samples or in the interval of waiting for samples to filter during the analysis.

Place 2.00 ml. ferrous solution, 2.00 ml. metaphosphoric acid solution and 2 drops indicator solution in a beaker and titrate with porphyrexide dye solution dropwise from a 5-ml. or 10-ml. burette calibrated in 0.05-ml. divisions. Endpoint is appearance of a pink color. Calculate dye strength as follows: 2.248 ÷ ml. titration = mg. ascorbic acid per ml. equivalent. The dye solution should be used only if the strength is in the range of 0.35 to 0.37 mg. per ml. equivalent.

Procedure: (a) Fluid products need no preparation. Dry milks should be reconstituted to the solids content of normal fluid milk. When analyzing dried products, it is important to reconstitute thoroughly and analyze immediately. (b) Pipette 10 ml. milk into a 250-ml. beaker and add 1.0 ml. indicator solution. Swirl and hold for 2.5 min. (c) Add 2.50 ml. porphyrexide dye solution dropwise from burette with swirling and hold for 10 min. (d) Add approximately 1 g. NaCl (dipper may be used) and swirl to dissolve. (e) Immediately add 5 ml. of H_2SO_4 solution while swirling and hold for 10 min. (f) Filter through Whatman no. 42 filter paper, pouring back the first 2 ml. of filtrate. Finally, procure at least 6 ml. filtrate for colorimetric measurement. (g) Transfer filtrate to Cenco 1.5-cm. absorption cells and read transmission. Use Cenco Photelometer, cat. no. 12346A with green (530 mμ) filter. Light intensity of photelometer should be set with water at 100 per cent transmission.

Calculations. From a standard chart read the mg. dye equivalent (as mg. ascorbic acid per liter) remaining in the milk after reaction with reducing substances. Subtract this value from mg. dye equivalents added to the milk (dye strength $\times 2.5 \times 100$) to obtain reducing groups in the milk expressed as mg. ascorbic acid equivalents per liter.

Standard chart. Expose raw milk in an open dish to direct sunlight for a few minutes to free of reduced ascorbic acid. To 10-ml. portions of the milk add 0-, 0.5-, 1.0-, 1.5-, 2.0- and 2.5-ml. portions of porphyrexide dye solution. Also, add sufficient water so amount of dye solution plus water equals 2.5 ml. Otherwise, follow the method procedure. Plot transmissions against mg. dye equivalents added per liter of milk (dye strength $\times$ ml. added to 10 ml. milk $\times 100$).

Correction for reduced ascorbic acid. The porphyrexide method measures not only heat-produced reducing substances, but also reduced ascorbic acid.

[2] Obtained from Eastman Kodak Co., Rochester, N. Y.

Therefore, to determine the amount of heat-produced reducing substances alone, a correction must be made. Reduced ascorbic acid may be determined by the method of Josephson and Doan (3) or Stewart and Sharp (17) and the value so obtained subtracted from the value obtained by the porphyrexide method.

Cooked flavor measurement. The standards for cooked flavor were made up by adding freshly sterilized evaporated milk (diluted 1:1) to HTST pasteurized milk. Ten parts of diluted evaporated milk to 90 of pasteurized milk was rated 1 for cooked flavor, etc., up to 60 diluted evaporated milk to 40 pasteurized, which was rated 6. The data used in table 2 were obtained from observations on samples of milk subjected to a variety of heat treatment conditions.

RESULTS

Insufficient data have been obtained to show the usefulness of the porphyrexide method to all phases of reducing substance studies. However, preliminary results indicate that it may prove a valuable tool. Some typical analyses are presented in table 1.

TABLE 1

Reducing substances in several milk products measured by the porphyrexide method

Sample		Reducing substances[a] per liter reconstituted product
Dry whole milk	#1	38
	#2	46
	#3	33
	#4	28
Nonfat dry milk, baking quality	#1	56
	#2	49
Nonfat dry milk, low heat	#1	22
	#2	25
Evaporated milk		118

[a] Expressed as mg. ascorbic acid.

The data contained in table 2 show that there is a good correlation between

TABLE 2

Cooked flavor scores and reducing substances measured by porphyrexide method on milk which had been subjected to various heat treatments

Cooked flavor score	No. of samples	Average reducing substances[a] per liter	Average deviation
0.5	5	12.5	0.4
1	9	22.6	2.5
1.5	13	26.3	2.3
2	14	34.3	2.3
2.5	8	36.9	1.4
3	3	41.3	0.9
3.5	2	44.3	2.2
4	2	48.3	0.3
4.5	3	56.7	1.8
5	2	64.0	0.5
6	1	77.5	

[a] Expressed as mg. ascorbic acid.

degree of cooked flavor with reducing substances measured by the porphyrexide method.

DISCUSSION

Advantages and limitations of the porphyrexide method are given as follows: (a) Reaction with reducing substances takes place at the normal pH of milk and at room temperature. (b) No reducing substances are measured in unheated milk except ascorbic acid. (c) The reaction is much more complete than when using indophenol dye. However, the reaction still cannot be considered as entirely complete when the method is used according to the directions given; in other words, the amount of dye reacting depends to some extent on the amount of excess dye present, although 10 min. time is sufficient to complete any reaction that is going to take place. (d) The method is not affected by concentrations of H_2SO_4 and NaCl in excess of that given in the directions. (e) The amount of p-aminodimethylaniline sulfate used is somewhat critical. The amount selected in the directions is based on obtaining complete reaction with excess porphyrexide. However, the indicator itself has a slight color and the amount used therefore must be kept constant. (f) The indicator solution is stable for at least 1 day, but the porphyrexide dye solution is relatively unstable and must be prepared and standardized at 1- to 4-hr. intervals. (g) Times given in the directions have been selected to allow complete reactions (porphyrexide with reducing substances and indicator, H_2SO_4 and NaCl with proteins) and times in excess have but little effect on the results. (h) Porphyrexide dye is difficult to synthesize and can be purchased commercially only at a rather high cost. This objection may be overcome if the method proves of sufficient value to merit its widespread use.

SUMMARY

A method has been developed for measuring heat-produced reducing substances in milk products. The method employs a powerful oxidizing dye, porphyrexide, in conjunction with an indicator, p-aminodimethylaniline sulfate. In preliminary tests its usefulness is indicated by its measurement of relatively large amounts of reducing substances in various milk products where heat is employed in processing. Other tests have shown the method to give results which correlate well with cooked flavor.

The porphyrexide method is offered for study and use by other investigators with the hope that it may serve as another tool in the study of the effects of heat on properties of milk.

REFERENCES

(1) Chapman, R. A., and McFarlane, W. D. A Colorimetric Method for the Estimation of Reducing Substances in Milk Powders. Can. J. Research, **23B**: 91–99. 1945.

(2) Crowe, L. K., Jenness, R., and Coulter, S. T. The Reducing Capacity of Milk and Milk Products as Measured by a Modified Ferricyanide Method. J. Dairy Sci., **31**: 595–610. 1948.

(3) Doan, F. J., and Josephson, D. V. Observations on the Ascorbic Acid Content of Evaporated Milk. J. Dairy Sci., **26**: 1031–1041. 1943.

(4) GOULD, I. A., AND SOMMER, H. H. Effect of Heat on Milk with Especial Reference to the Cooked Flavor. Mich. Agr. Expt. Sta. Tech. Bull. 164. 1939.

(5) GREENSTEIN, J. P. Sulfhydryl Groups in Proteins. I. Egg Albumin in Solutions of Urea, Guanidine, and their Derivatives. J. Biol. Chem., **125**: 501–513. 1938.

(6) HARLAND, H. A., AND ASHWORTH, U. S. The Use of Thiamin Disulfide for the Estimation of Reducing Substances in Processed Milk. J. Dairy Sci., **28**: 15–23. 1945.

(7) HARLAND, H. A., COULTER, S. T., AND JENNESS, R. Some Relationships of the Oxidation-reduction Systems of Milk to the Keeping Quality of the Dry Product (Abs.). J. Dairy Sci., **33**: 408. 1950.

(8) JACKSON, C. J. Note on the Sulphydryl Compounds of Milk. J. Dairy Research, **7**: 29–30. 1936.

(9) JOSEPHSON, D. V., AND DOAN, F. J. Observations on Cooked Flavor in Milk: Its Sources and Significance. Milk Dealer, **29**, 2: 35. 1939.

(10) KUHN, R., AND DESNUELLE, P. Zur Bestimmung von Sulfhydrylgruppen in Proteinen. Z. physiol. Chem., **251**: 14–18. 1938.

(11) KUHN, R., AND FRANKE, W. T. Über das Redox-potential des Porphyrexids und des Porphyrindins. Ber., **68B**: 1528–1536. 1935.

(12) LARSEN, R. A., JENNESS, R., AND GEDDES, W. F. Effect of Heat Treatment on the Sulfhydryl Groups of Milk Serum Proteins. Cereal Chem., **26**: 287–297. 1949.

(13) LILLEVIK, H. A., HOSSFELD, R. L., LINDSTROM, H. V., ARNOLD, R. T., AND GORTNER, R. A. Technique in the Synthesis of Porphyrindine. J. Org. Chem., **7**: 164–168. 1942.

(14) PILOTY, O., AND SCHWERIN, B. Über das Nitril der Nitrosoisobuttersäure und seine Derivate. Ber., **34**: 1863–1870. 1901.

(15) PORTER, C. C., AND HELLERMAN, L. Porphyrexide and Porphyrindine Analogs Derived from 1-Hydroxyaminocyclohexyl Cyanide. J. Am. Chem. Soc., **66**: 1652–1655. 1944.

(16) STEWART, A. P., JR. The Effect of the Interaction of Casein and Lactose on Certain Properties of Milk and Milk Products. Ph.D. Thesis, Cornell University. 1942.

(17) STEWART, A. P., JR., AND SHARP, P. F. Determination of Vitamin C in the Presence of Interfering Reducing Substances. Selective Oxidation-reduction Method. Ind. Eng. Chem., Anal. Ed., **17**: 373–376. 1945.

VITAMIN B_{12} IN BLOOD OF NEWBORN AND COLOSTRUM-FED CALVES AND IN COLOSTRUM AND NORMAL MILK OF HOLSTEIN AND JERSEY COWS[1, 2]

W. B. ANTHONY,[3] J. R. COUCH,[4] I. W. RUPEL,[3] WITH THE TECHNICAL ASSISTANCE OF MARY B. HENDERSON AND CHARLESTA BROWN

Agricultural and Mechanical College of Texas and Texas Agricultural Experiment Station, College Station

Studies on the levels of various blood constituents have yielded considerable information relative to the dietary requirements and health of the young calf. Such records before and following ingestion of colostrum have helped define its role in establishing the normal blood picture in calves. Pearson and Darnell (4) reported on the concentration of thiamin, riboflavin, niacin and pantothenic acid in colostrum from Holstein and Jersey breeds. Sutton (6, 7), Hand (2), Johnson (3), Theophilus (8) and Whitnah (9) with their respective co-workers have reported on the levels of riboflavin in colostrum and factors affecting its level in milk. To the authors' knowledge, however, no information has been published concerning the concentration of vitamin B_{12} in colostrum or in the blood of the dairy calf at birth and following the ingestion of colostrum. Accordingly, this investigation was carried out to determine the vitamin B_{12} in colostrum and in blood of calves.

EXPERIMENTAL PROCEDURE

Colostrum and milk samples were collected from purebred Holstein and Jersey cows calving in the A. & M. College herd during July, August and September, 1950. The feeding regime for this herd consisted of a 15 to 16 per cent protein grain mixture, alfalfa hay, sorghum silage and some sudan-grass pasture. A few of the samples were from first-calf heifers but most of the samples were from older cows which freshened after a rest period of 6 to 8 wk.

The cows were machine-milked, and a 0.5-pt. sample was taken immediately from the milking machine bucket. The first milking always occurred within 6 hr. after the cow calved. The other samples were taken 24, 48, 72, 96, 144 and 192 hr., and 15, 30, 45 and 60 days after calving. Calves were allowed to remain with their dams for the first day and, consequently, the first milking reported in this study represents milk remaining in the udders after calves had nursed.

In most instances, vitamin B_{12} analysis was begun a few hours after the samples were collected. If the analysis was delayed, samples were frozen immediately after collection and stored until processed.

Received for publication Feb. 15, 1951.

[1] Supported in part by Project RC 1862, Division of Research Grants and Fellowships, U. S. Public Health Service, National Institute of Health, Bethesda, Maryland.

[2] The crystalline vitamin B_{12} used in the microbiological assays was generously supplied by D. F. Green, Veterinary Department, Merck and Co., Rahway, New Jersey.

[3] Department of Dairy Husbandry.

[4] Biochemistry and Nutrition and Poultry Husbandry Departments.

Stock solutions were prepared for microbiological assay as indicated by Couch *et al.* (1). Ten ml. of colostrum or milk (in the case of the blood samples, 3 ml. of oxalated whole blood) were pipetted into 50 ml. of water in a 125-ml. flask. Ten ml. of a pancreatin solution (3 mg. per milliliter) were added and the solution was covered with toluene. The cork-stoppered flask was shaken at 37.5° C. for approximately 24 hr. The samples then were autoclaved for 5 min. at 15 lb. pressure. After cooling, the samples were made up to 100 ml. and filtered. The filtrates were covered with toluene and stored at approximately 20° C. The filtrates constituted the stock solutions from which dilutions for the microbiological assays for vitamin B_{12} were made. These were made according to the procedure of Skeggs *et al.* (5), with *Lactobacillus leichmannii* 4797 (ATCC) as the test organism, and acid production was the criterion used to measure growth response. Crystalline vitamin B_{12} was used as the standard in all of the assays mentioned in this report.

The calves in this study were dropped by the cows from which the colostrum and milk samples were taken. Blood samples were drawn from the jugular vein immediately following birth and before ingestion of colostrum. Additional blood samples were taken 24, 48, 72, 96, 144 and 192 hr. after birth. Each calf was hand-fed the milk from its dam after the first day of life for 4 days. After the fifth day, mixed milk from Holstein cows was fed. Data were collected on seven Holstein and six Jersey calves. The number of samples at respective ages is less than the total number of calves due to loss of certain samples and the fact that certain calves were unavoidably omitted at given ages.

RESULTS

Vitamin B_{12} values in the blood of Holstein and Jersey calves at birth and following the ingestion of colostrum are presented in table 1. In contrast to

TABLE 1

Vitamin B_{12} in blood of dairy calves before and following ingestion of colostrum

Age (*hr.*)	<6	24	48	72	96	144	192
			Holstein calves				
No. sampled this age	5	5	5	6	4	6	6
Range (mμg./ml.)	1.0–1.2	0.9–1.7	0.6–1.2	0.6–1.9	0.7–1.4	0.8–1.6	0.7–1.3
Mean (mμg./ml.)	1.1	1.2	1.0	1.1	1.0	1.2	1.1
			Jersey calves				
No. sampled this age	5	5	4	4	4	4	4
Range (mμg./ml.)	0.7–1.3	0.9–1.4	0.6–1.6	0.7–1.5	0.7–1.1	0.4–1.7	0.5–1.3
Mean (mμg./ml.)	1.0	1.1	1.1	1.1	0.8	1.1	0.9

changes of some other constituents of blood of young calves following the ingestion of colostrum, these data indicate a normal blood level of vitamin B_{12} for the dairy calf at birth. No increase was shown in blood B_{12} following the ingestion of colostrum. By referring to table 2, one may observe the vitamin B_{12} content of the colostrum consumed by the calves from which the data in table 1 was

collected. Since only one nutritional level was studied, these data, therefore, do not clearly indicate placental transfer of vitamin B_{12}.

The results of analyses for vitamin B_{12} in colostrum and normal milk from purebred Holstein and Jersey cows are shown in table 2. Samples were collected from 15 Jerseys and 16 Holsteins in the College herd. The colostrum was sampled within 6 hr. following calving. The colostrum and milk were followed by sampling at 24, 48, 72, 144, 192 hr., and 15, 30 and 60 days after calving to determine the vitamin B_{12} content. A few samples were lost before the assays were completed and some cows were unavoidably missed at given times. Hence, the number of assays completed was lower at certain intervals after calving than the total number of cows included in the study, but the data indicate something of the individual variability and trend following calving. The high initial concentration of vitamin B_{12} in colostrum from Holstein cows was greater, in most instances, than the concentration found in Jersey colostrum. Although varia-

TABLE 2

Vitamin B_{12} in colostrum and normal milk from Holstein and Jersey cows

Time after calving	Holstein cows			Jersey cows		
	No. assays	Range	Mean	No. assays	Range	Mean
		($m\mu g./ml.$)	($m\mu g./ml.$)		($m\mu g./ml.$)	($m\mu g./ml.$)
< 6 hr.	13	28–78	49 ± 5.4	13	3–56	21 ± 4.6
24 "	16	15–40	25 ± 2.3	12	2–35	18 ± 2.7
48 "	14	12–37	24 ± 2.2	13	4–40	17 ± 2.9
72 "	12	13–45	27 ± 3.0	12	8–27	16 ± 1.3
144 "	14	14–48	25 ± 2.5	14	4–27	14 ± 2.0
8 d.	12	11–38	22 ± 2.5	13	3–25	10 ± 1.6
15 "	6	5–36	16 ± 4.4	6	3–15	7 ± 1.7
30 "	10	3–24	10 ± 1.9	9	2–14	7 ± 1.4
45 "	11	3–15	9 ± 1.0	12	2–11	7 ± 0.9
60 "	10	2–9	5.9 ± 0.6	14	2–13	5.6 ± 0.8

tions have been reported in vitamin content of colostrum from different breeds (2, 7), the differences found in this experiment were more pronounced.

While the daily decline in vitamin B_{12} was rapid in the Holstein colostrum, on the eighth day the level remained as high as that present initially in average Jersey colostrum. Although the ranges of the two breeds overlap considerably, the means (at 144 hr.) are significantly different at the 5 per cent level of probability.

These data showing a greater concentration of vitamin B_{12} in the colostrum and milk from the Holstein than in corresponding secretions from the Jersey are in contrast to findings with respect to other B vitamins in normal milk (2, 3, 6). The breed differences observed are not explained by the data from this work.

The milk from 48 Jersey cows was sampled separately at the a.m. and p.m. milkings and analyzed for its vitamin B_{12} content. At the time of sampling, the cows were fresh from 6 to 351 days, averaging 139 days, the group thus furnishing a more or less typical sample of normal herd milk.

TABLE 3

Vitamin B_{12} content of normal Jersey herd milk

Time milked	No. samples	Range, B_{12}	Mean
		(*mμg./ml.*)	(*mμg./ml.*)
A. M.	48	3–22	8.7 ± 0.63
P. M.	48	4–13	6.9 ± 0.33
Mean for day	48	4–16	7.6 ± 0.47

The mean of the morning values was 8.7 millimicrograms per milliliter of milk, while the evening milkings averaged 6.9, a difference of 1.8 millimicrograms. Analysis of variance showed this to be significant at the 5 per cent level of probability. The reason for the difference between a.m. and p.m. values is not apparent. It will be noted that the mean of 7.6 millimicrograms (daily averages, 48 cows) is somewhat higher than the means at 60 days shown in table 2. The samples in table 2 represent single milkings and the smaller numbers may merely reflect sampling differences.

SUMMARY

Vitamin B_{12} determinations were made on the blood of calves before ingestion of colostrum and subsequently to 8 days of age. No significant differences were observed in relation to age (within this range), breed or the food ingested.

Holstein colostrum showed nearly double the values observed on Jersey colostrum. The colostrum B_{12} values for both breeds declined gradually. The Jersey milk, showing averages of 7.0 millimicrograms after the 15th day, appeared to have declined to "herd average" levels. The Holstein milk maintained a slightly higher value through the 45th day.

Forty-eight Jersey cows fresh from 6 to 351 days showed an average vitamin B_{12} value of 7.6 millimicrograms per milliliter. Morning milk averaged 8.7 *versus* 6.9 for evening milk (with significance at the 0.05 level).

ACKNOWLEDGMENT

The authors wish to extend their sincere appreciation to James Huff and Claude Free for assistance in collecting many of the blood and colostrum samples for this experiment.

REFERENCES

(1) Couch, J. R., Olcese, O., Witten, P. W., and Colby, R. W. The Vitamin B_{12} Content of Blood from Various Species. Am. J. Physiol., **163**: 77–80. 1950.

(2) Hand, D. B., and Sharp, P. F. The Riboflavin Content of Cow's Milk. J. Dairy Sci., **22**: 779–783. 1939.

(3) Johnson, P., Maynard, L. A., and Loosli, J. K. The Riboflavin Content of Milk as Influenced by Diet. J. Dairy Sci., **24**: 57–64. 1941.

(4) Pearson, P. B., and Darnell, A. L. The Thiamine, Riboflavin, Nicotinic Acid, and Pantothenic Acid Content of Colostrum and Milk of the Cow and Ewe. J. Nutrition, **31**: 51–58. 1946.

(5) Skeggs, Helen R., Nepple, Helga M., Valentik, Katherine A., Huff, Jesse W., and Wright, L. D. Observations on the use of *Lactobacillus leichmannii* 4797 in the Microbiological Assay of Vitamin B_{12}. J. Biol. Chem., **184**: 211–221. 1950.

(6) SUTTON, T. S., AND KAESER, H. E. The Riboflavin Content of Cows' Colostrum. J. Dairy Sci.. **30**: 581–582. 1947.

(7) SUTTON, T. S., WARNER, R. G., AND KAESER, H. E. The Concentration and Output of Carotenoid Pigments, Vitamin A, and Riboflavin in the Colostrum and Milk of Dairy Cows. J. Dairy Sci., **30**: 927–932. 1947.

(8) THEOPHILUS, D. R., AND STAMBERG, O. E. The Influence of Breed, Feed, and Processing on the Riboflavin Content of Milk. J. Dairy Sci., **28**: 259–268. 1945.

(9) WHITNAH, C. H., KUNERTH, B. L., AND KRAMER, M. M. Riboflavin Content of Milk Collected in Different Months, and Correlated with Other Constituents of the Milk. J. Dairy Sci., **21**: 593–600. 1938.

ENUMERATION OF LACTIC STREPTOCOCCUS BACTERIOPHAGE BY A TWO-LAYER PLATE METHOD FOR PLAQUES[1]

G. E. TURNER[2] AND F. E. NELSON

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The adoption of a procedure that will enumerate at least a fairly definite proportion of the absolute numbers of bacteriophage particles present is a fundamental requirement for study of bacteriophage activity. Of the methods used to obtain estimates of lactic streptococcus (*Streptococcus lactis* and *Streptococcus cremoris*) bacteriophage populations, the two commonly employed are (a) determination of the limiting dilution of bacteriophage which can cause marked delay in reduction and acid production in litmus milk containing susceptible bacteria and (b) enumeration of lysed areas or "plaques" formed when a mixture composed of susceptible cells and a dilution of bacteriophage suspension is smeared upon a hardened agar surface and the resultant plate incubated under suitable conditions. Cherry and Watson (6) have devised a two-layer agar plate method for counting plaques that has a number of points in common with the method reported here; their procedure was applied to only one lactic streptococcus-bacteriophage combination.

For enumerating bacteriophage particles by plaque formation, Yen (18) considered that the bacteriophage particles and organisms would be more uniformly distributed within a single layer of agar medium than in a smear of a similar mixture over the surface of a solid medium. Difficulty was experienced in obtaining clearly defined plaques at different levels within the single agar layer. This difficulty was overcome by Gratia (11) who employed a solid agar base upon which was placed a thin agar overlay containing the bacteriophage and organism. A number of reports (2, 3, 4, 6, 10, 13, 14, 18) have shown that the number of plaques obtained from a given bacteriophage suspension may vary considerably as the result of changes in the details of the enumeration technic. Increasing the agar concentration and/or the thickness of the agar layer (3, 4, 12, 14) materially reduced the number of countable plaques. Variations in amount of the bacterial suspension added to the petri plate were reported by some (4, 14) to be without effect on plaque formation, whereas others (5, 6, 18) have indicated that a critical cell concentration is desirable for maximum plaque formation.

The present study was undertaken, first, to ascertain whether the factors reported to affect plaque formation by other bacteriophages influenced plaque formation by lactic streptococcus bacteriophage and, second, to develop a plaque plate technic suitable for enumerating bacteriophage active against lactic streptococci.

Received for publication Feb. 16, 1951.

[1] Journal paper no. J-1440 of the Iowa Agricultural Experiment Station, Ames. Project no. 652.

[2] Present address: Dept. of Dairy Manufactures, National Agricultural College, Doylestown, Penn.

MATERIALS AND METHODS

The five bacteriophage-organism (*Streptococcus cremoris*) combinations studied were obtained from the collection maintained in the Dairy Bacteriology Laboratories at Iowa State College and represented combinations from England, New Zealand, Canada and the United States. All produced well-defined plaques using a two-layer agar plate method patterned after that which Delbrück (9) employed to study proliferation of coliform bacteriophage. In the early studies approximately 12 ml. of sterile tomato juice-peptonized milk medium of 1 per cent agar strength were used in the lower layer, with an agar overlay containing 1 ml. of 1:100 aqueous dilution of susceptible cells, 1 ml. of bacteriophage suspended in water and 2 ml. of the same tomato juice agar as used for the basal layer, added in the order mentioned and mixed well before hardening. The plates were incubated in an upright position at 30° C. for approximately 10 hr.

After study of the various factors that affect plaque formation and enumeration, the preliminary procedure was modified as follows: 1 ml. of 1:25 aqueous dilution of susceptible cells from a milk culture which had just begun to coagulate, 1 ml. of sterile skimmilk, containing the diluted bacteriophage and 1 ml. of the agar medium containing 1.5 per cent agar (in place of the original 1.0 per cent concentration) were used for the agar overlay, the different materials being combined in the order mentioned. Thus, the final agar concentration of the overlay remained at 0.5 per cent, whereas the volume was reduced from 4 to 3 ml. Figure 1 shows developed plaques appearing on plates prepared by this

FIG. 1. Plaques on plate prepared with bacteriophage PF11 and organism HI-10.

technic. Some of the plaques illustrated are larger than those obtained with certain other strains of bacteriophage. Agar for the overlay was measured accurately because the concentration and the thickness of agar in the overlay were found to affect the number of plaques appearing on the plates. Agar for the overlay should be at 55° C. just before pipetting into the 2 ml. of material already in the plate, as excessive cooling of the medium results in a granular appearance of the agar and poorly defined plaques.

The tomato juice-peptonized milk agar was prepared by dissolving 10 g. peptonized milk, 10 g. proteose peptone and either 10 or 15 g. of agar in distilled water and making the volume to 600 ml. with distilled water. To this was added 400 ml. of clear tomato juice, previously adjusted colorimetrically to pH 6.4. After the influence of medium reaction on plaque formation had been demonstrated, the agar always was used at pH 5.8 to 6.0, any necessary adjustment being made after sterilization.

When the limiting dilution technic for phage particle enumeration (15) was used, the required dilutions were added to 8-ml. quantities of sterile litmus milk containing 0.1 ml. of active susceptible culture; incubation was at 30° C. for 18 to 20 hr. Marked retardation in the appearance of normal reduction, acid and coagulation was considered indicative of the presence of bacteriophage in the material tested. When considerable accuracy was desired, five tubes of each dilution were prepared and the most probable number of bacteriophage particles estimated from probability tables (1).

Whey ultra-filtrates from bacteriophage-lysed cultures in milk were used as stock bacteriophage preparations. Occasional repropagations kept the bacteriophage preparations at high titers.

To control the variable of the physiological state of the test bacteria and to assist in controlling the numbers of bacteria added, the bacterial cultures always were incubated at 30° C. until coagulation was observed. The length of the incubation period and the count per milliliter of culture, using tomato juice-peptonized milk agar and 48-hr. incubation at 30 to 32° C., were used as bases for subsequent trials. By following this procedure for all five cultures used in this study, it was possible to dispense with a daily direct microscopic count of cell numbers and every culture also was considered to be in a similar physiological state.

RESULTS

Influence of agar concentration and thickness of agar overlay on plaque formation. The preliminary two-layer method was used, except that simultaneous increases in agar concentration and thickness of the overlay were obtained by adding increasing volumes of 1 per cent agar to the overlay. The results of three trials with PF11 bacteriophage indicated that simultaneous increases in agar concentration and thickness of the overlay reduced the plaque count to 57 and 46 per cent when the agar concentration was increased to 0.60 and 0.67 per cent, respectively, as compared to the count obtained when the concentration was 0.50 per cent. Table 1 presents data on five bacteriophage strains

TABLE 1

The influence of volume of agar overlay on number and size of bacteriophage plaques

Bacteriophage-organism combination[a]	Bacteriophage diln. used	Agar volume[b]					
		4 ml.		3 ml.		2 ml.	
		Plaque count[c]	Av. plaque size	Plaque count	Av. plaque size	Plaque count	Av. plaque size
			(*mm.*)		(*mm.*)		(*mm.*)
F69/ML1	10^{-5}	154	1.25	170	1.75	96	1.75
F68/IP5	10^{-6}	136	1.25	156	1.50	110	1.50
F70/SH5	10^{-4}	92	1.50	158	2.00	139	1.25
PF2/H1–2	10^{-7}	123	1.50	120	1.50	40	0.75
PF11/H1–10	10^{-7}	71	1.50	108	1.00	22	1.00

[a] The first designation refers to the bacteriophage strain and the second to the organism strain.

[b] Agar concentration of top layer was 0.5% in all plates.

[c] Total plaques on duplicate plates.

when the final agar concentration of the overlay was maintained at 0.5 per cent while the thickness of the agar layer was varied by using total volumes of 2, 3 and 4 ml. for the second layer. When the total volume of the agar overlay was 3 ml., maximum plaque counts were obtained for all bacteriophage strains studied, except for PF2. The difference in results between 3 and 4 ml. overlays for PF2 undoubtedly was not significant. The plaque sizes varied with changes in the thickness of the agar layer, but no definite trends were indicated, although the plaques obtained with the 3-ml. total volume were as large as or larger, except with PF11, than those secured with the other two volumes.

Influence of the concentration of susceptible cells added to the agar overlay on plaque formation. A plate count of approximately 12 million was obtained on the amount of culture added to the agar overlay in the preliminary studies. In further studies plate counts of approximately 0.07, 0.7, 7, 14, 21, 28 and 35 million per 3 ml. of agar overlay were tried. Data in table 2 showed that the greatest numbers of plaques were obtained when the plate counts of bacteria in the agar overlay were in the range from 21 to 35 million. As a result of these findings, the plate count of the bacterial culture thereafter added to the agar overlay approximated 28 million.

TABLE 2

The influence of concentration of susceptible cells in the agar overlay on plaque numbers

Bacteriophage-organism combination	Bacteriophage diln. used	Total plaques on 3 replicate plates with approximately the following plate count in the agar overlay						
		0.07M[a]	0.7M	7M	14M	21M	28M	35M
F69/ML2	10^{-5}	46	86	185	167	224	261	191
F68/IP5	10^{-6}	6	59	138	161	181	246	182
F70/SH5	10^{-7}	4	53	81	172	197	213	217
PF2/H1–2	10^{-7}	15	53	138	145	165	160	159
PF11/H1–10	10^{-7}	1	29	69	134	131	135	123

[a] M = Millions.

Influence of milk added to the agar overlay on plaque numbers. To study this factor, dilutions of all the bacteriophage strains under study were prepared in both milk and water. One ml. of the milk dilution of bacteriophage was added to each of two or three petri plates, after which 1 ml. of susceptible organism suspended in water and 1 ml. of 1.5 per cent agar medium were added. The water suspension of bacteriophage was handled similarly. Two or three additional plates were prepared from the bacteriophage suspended in water, but 1 ml. of susceptible cells in milk was added to teach plate in place of the customary 1 ml. of water-suspended cells. Data in table 3 indicate that using water as a

TABLE 3

The effect of milk in the agar overlay on plaque numbers

Bacteriophage-organism combination	Bacteriophage diln. used	No. plates in each group	Total plaques on plates from dilutions made in:		
			Water[a]	Milk[b]	Water & milk[c]
F69/ML2	10^{-7}	3	70	179	181
F68/IP5	10^{-7}	2	99	92	102
F70/SH5	10^{-7}	3	3	30	27
PF2/H1–2	10^{-7}	2	75	69	72
PF11/H1–10	10^{-7}	2	22	58	59

[a] Both the bacteriophage and the susceptible cells were dispersed in water and no milk was present in the 3-ml. second layer.

[b] 1 ml. of milk was present in the 3-ml. second layer.

[c] The susceptible bacterial cells were added to sterile milk in place of the usual water; 1 ml. of milk was present in the 3-ml. second layer.

bacteriophage diluent in place of milk caused drastic reduction in the plaque count of three of the bacteriophage strains; addition of milk to the water dilution of bacteriophage contained in the petri plate restored the plaque count to a level equal to that obtained when the bacteriophage was suspended directly in milk. Two of the five bacteriophage strains were not affected in this manner when diluted with water. Whey, tomato juice-peptonized milk broth and phosphate-buffered water could not be susbstituted satisfactorily for milk as a dilution medium, when bacteriophage PF11 was used for testing. As a result of these findings, milk instead of water was used for preparing the final dilution of bacteriophage preparations for plating.

Lengthening the time of heat treatment of the milk incorporated into the agar layer from 20 to 45 min. resulted in 40 and 45 per cent reductions in the plaque count in two trials using bacteriophage PF11 for testing. Caramelization of the milk took place at 15 lb. pressure for 20 min. and definite browning was evident when milk was held for 45 min. at the same pressure. As a result of these findings, the milk used for diluting bacteriophage particles was sterilized at 15 lb. steam pressure for 15 min.

Influence of the pH of the plating medium on plaque formation. During the first few trials using the two-layer agar method, the plating medium was adjusted to pH 7.2 just prior to sterilization and no further adjustment was made. In subsequent studies, the pH of the plating medium was varied after sterilization and it was found that on four plates at each pH level, bacteriophage strain

PF2 produced 143 plaques when the reaction of the plating medium was pH 7.2, 512 plaques at pH 6.0, 282 plaques at pH 5.4 and 4 plaques at pH 5.2. No plaques were obtained when the medium was above pH 8 or below pH 5. For further study, agars at different pH levels were prepared for use in both basal and overlay layers and ten plates were poured for each bacteriophage-organism combination at each pH level. The results obtained are given in table 4. With

TABLE 4

The influence of pH of the agar plating medium on the plaque counts

Bacteriophage-organism combination	Bacteriophage diln. used	Total plaques on 10 replicate plates at pH:							
		7.2	6.4	6.2	6.0	5.8	5.6	5.4	5.2
F69/ML1	10^{-7}	117 (40.2)[a]	232 (79.7)	254 (87.3)	290 (99.6)	291 (100)	283 (97.3)	237 (81.4)	18 (6.2)
F68/IP5	10^{-7}	462 (56.9)	661 (81.5)	666 (82.1)	811 (100)	721 (88.9)	717 (88.4)	573 (70.6)	364 (44.9)
F70/SH5	10^{-8}	96 (45.7)	149 (70.9)	177 (84.3)	178 (84.8)	210 (100)	149 (70.9)	47 (22.4)	7 (3.3)
PF2/H1–2	10^{-7}	143 (27.4)	210 (40.3)	429 (82.3)	521 (100)	470 (90.2)	482 (92.5)	282 (54.1)	17 (3.1)
PF11/H1–10	10^{-7}	168 (47.4)	262 (74.0)	286 (81.0)	354 (100)	291 (82.2)	298 (84.2)	228 (81.4)	11 (3.1)

[a] Figures in parenthesis indicate per cent of maximum for that particular series.

all combinations, a pH level of 5.8 to 6.0 gave the maximum number of plaques. As the pH was increased, the plaque count decreased until at pH 7.2 plaque counts ranged from 27 to 57 per cent of the maximum. Decreasing the pH to 5.2 reduced the plaque count to less than 7 per cent of the maximum in four cases out of five studied. As a result of these findings, the tomato juice-peptonized milk agar always was used at pH 5.8 to 6.0, the reaction being adjusted after sterilization, if necessary.

Comparison between the plaque plate and litmus milk dilution methods for measuring bacteriophage activity. To make this comparison with freshly prepared bacteriophage, triplicate plaque plates and five tubes of litmus milk for each dilution were used. This determination was repeated 10 and 25 days later. The whey filtrates were stored at approximately 5° C. during the period of study. The results (table 5) indicated that while either method may be used to deter-

TABLE 5

Comparison of bacteriophage enumeration by plaque count and litmus milk dilution technics

Bacteriophage-organism combination	Counts per ml.[a] after storage at 5° C. for:					
	1 d.		10 d.		25 d.	
	Plaque count	MPN	Plaque count	MPN	Plaque count	MPN
F69/ML1	30×10^7	95×10^6	19×10^6	13×10^6	10×10^5	79×10^4
F68/IP5	45×10^7	14×10^7	12×10^7	49×10^6	62×10^6	33×10^6
F70/SH5	91×10^6	70×10^6	28×10^6	28×10^6	46×10^5	23×10^5
PF2/H1–2	29×10^8	24×10^8	21×10^8	79×10^7	94×10^7	24×10^7
PF11/H1–10	35×10^8	24×10^8	14×10^8	79×10^7	53×10^7	35×10^7

[a] Count based upon plaque counts (av. of 3 plates) or on most probable number (MPN) per ml. based on 5 tubes per dilution.

mine bacteriophage activity, the plaque plate method gave results that were slightly higher in every comparison, except for one case in which the results were identical.

DISCUSSION

These studies emphasize the need for maintaining optimum conditions and for employing a rigid procedure in using the plaque plating method to enumerate lactic streptococcus bacteriophage particles. The claims of some investigators (3, 13, 15) that reduced plaque formation results from increases in agar concentration and thickness of agar layer are corroborated in this study. To secure maximum plaque formation, an optimum cell concentration in the agar overlay is necessary, confirming the work of Burnet (5). The count of host cells found optimum agrees with that reported by Cherry and Watson (6). Presumably, low concentrations of added cells give an insufficient available concentration of cells to which bacteriophage particles may adsorb, resulting in lower plaque counts because of lack of suitable substratum for initiation of bacteriophage proliferation. Increasing the cell concentration probably increases the chance that the particle will adsorb to the cell by decreasing the distance between the bacterial cell and bacteriophage particle. Other results from this laboratory indicate that the forces of attraction between the cell and particle may not be active over an appreciable distance.

For three of the five combinations of organism and bacteriophage, addition of milk to the top layer gives a very considerable increase in countable plaques. Subsequent to the completion of these studies, Shew (17) reported that eight strains of bacteriophage active against *S. lactis* all needed calcium for maximum development, as judged by plaque formation on solid media and clearing in broths. Reiter (16) used 0.01 *M* $CaCl_2$ in his agar medium for demonstrating plaque formation by bacteriophages from three lactic streptococci. Cherry and Watson (7) reported $CaCl_2$ was stimulatory to proliferation of one strain of lactic streptococcus bacteriophage studied on a yeast extract-tryptone-glucose medium. Collins *et al.* (8) have shown that soluble calcium is essential for proliferation in a defined medium by eight of ten lactic streptococcus bacteriophages. These results indicate the possibility that the presence of milk in the plates may be beneficial because of the calcium which is contributed. Results now being obtained in this laboratory indicate that calcium is the factor, and a report on these studies will be presented in a subsequent paper.

The method developed in this study gives results higher than those obtained by the dilution procedure, in contrast to the procedure of Cherry and Watson (6) which gave plaque counts which were one-fourth to one-twentieth of the most probable number of bacteriophage particles as determined by a limiting dilution technic. The tendency for bacteriophage population estimates to be higher by this method indicates that the plaque plate method may give a truer picture of the population. However, certain bacteriophage strains failed to produce countable plaques, even under the best conditions employed in this study. Selection of five strains of bacteriophage which could be enumerated reasonably well by the plaque procedure was considered justifiable because of the greater

possible accuracy and convenience of the plaque enumeration method for study of certain factors concerned in reproduction of bacteriophage. To enumerate strains of bacteriophage that produce poorly defined plaques, one apparently has no alternative at present but to use the dilution methods or to devise additional modifications of plaque enumeration procedures.

SUMMARY AND CONCLUSIONS

A two-layer agar plate technic has been developed for enumeration of lactic streptococcus bacteriophage particles. Upon a basal layer of 12 ml. of tomato-peptonized milk medium of 1.0 per cent agar concentration which has been allowed to harden in a petri plate is superimposed an overlay consisting of 1 ml. of aqueous suspension of susceptible bacterial cells (plate count approximately 28 million), 1 ml. of a suitable milk dilution of bacteriophage and 1 ml. of the above plating medium containing 1.5 per cent agar. Incubation at 30° C. produced countable plaques in 8 to 10 hr.

Among the factors shown to influence the results obtained by this procedure were volume and agar concentration of the overlay, count of bacteria added, pH of the medium and inclusion of milk in the overlay.

REFERENCES

(1) American Public Health Association. *Standard Methods for the Examination of Dairy Products*, 9th ed. Am. Pub. Health Assoc., New York, N. Y. 1948.

(2) Anderson, T. F. The Influence of Temperature and Nutrients on Plaque Formation by Bacteriophages Active on *Escherichia coli* Strain B. J. Bact., **55**: 659–665. 1948.

(3) Bronfenbrenner, J., and Korb, C. On the Factors Influencing the Appearance of Plaques of Bacterial Lysis. Proc. Soc. Exptl. Biol. Med., **21**: 315–316. 1923.

(4) Bronfenbrenner, J., and Korb, C. Studies on the Bacteriophage of d'Herrelle. III. Some of the Factors Determining the Number and Size of Plaques of Bacterial Lysis. J. Exptl. Med., **43**: 483–498. 1925.

(5) Burnet, F. M. The Conditions Governing the Appearance of Taches Vierges in Bacteriophage Activity. J. Path. and Bact., **28**: 419–425. 1925.

(6) Cherry, W. B., and Watson, D. W. The *Streptococcus lactis* Host-virus System. I. Factors Influencing Quantitative Measurement of the Virus. J. Bact., **58**: 601–610. 1949.

(7) Cherry, W. B., and Watson, D. W. The *Streptococcus lactis* Host-virus System. II. Characteristics of Virus Growth and the Effect of Electrolytes on Virus Adsorption. J. Bact., **58**: 611–620. 1949.

(8) Collins, E. B., Nelson, F. E., and Parmelee, C. E. Relation of Calcium and Other Constituents of a Defined Medium to Proliferation of Lactic Streptococcus Bacteriophage. J. Bact., **60**: 533–542. 1950.

(9) Delbrück, M. The Burst Size in the Growth of Bacterial Viruses (Bacteriophages). J. Bact., **50**: 131–135. 1945.

(10) Dreyer, G., and Campbell-Renton, Margaret L. The Quantitative Determination of Bacteriophage Activity and its Application to the Study of the Twort-d'Herelle Phenomenon. J. Path. and Bact., **36**: 399–423. 1933.

(11) Gratia, A. Des relations numeriques entre bacteries lysogenes et particules de bacteriophage. Ann. Inst. Pasteur, **57**: 652–676. 1936.

(12) Hershey, A. D., and Bronfenbrenner, J. The Influence of Host Resistance on Virus Infectivity as Exemplified with Bacteriophage. J. Gen. Physiol., **24**: 703–707. 1941.

(13) HERSHEY, A. D., KALMANSON, G., AND BRONFENBRENNER, J. Quantitative Methods in the Study of the Phage-antiphage Reaction. J. Immunol., **46**: 267–280. 1943.

(14) KLECKOWSKA, J. A Quantitative Study of the Interaction of Bacteriophage with Rhizobium Using the Technique of Poured Plates. J. Bact., **50**: 81–94. 1945.

(15) NELSON, F. E., HARRIMAN, L. A., AND HAMMER, B. W. Slow Acid Production in Butter Cultures. Iowa Agr. Expt. Sta. Research Bull. 256. 1939.

(16) REITER, B. Lysogenic Strains of Lactic Streptococci. Nature (London), **164**: 667–668. 1949.

(17) SHEW, D. I. Effect of Calcium on the Development of Streptococcal Bacteriophages. Nature (London), **164**: 492–493. 1949.

(18) YEN, A. C. H. Pour Plate Study of Bacteriophage. Proc. Soc. Exptl. Biol. Med., **32**: 1006–1010. 1935.

THE FERTILITY OF BOVINE SEMEN IN DILUTERS CONTAINING VARYING AMOUNTS OF EGG YOLK[1]

JOHN O. ALMQUIST

Dairy Cattle Breeding Research Center, Pennsylvania Agricultural Experiment Station, State College

Until recently most of the bovine semen diluters used in this country have consisted of a mixture of one part egg yolk to one part buffer, as recommended in 1940 by Phillips and Lardy (6). In 1949, Swanson (8) reported that the amount of egg yolk could be reduced from 50 to 20 per cent in the presence of an isotonic buffer solution without impairing spermatozoan livability. Lowering the concentration of egg yolk to 10 per cent reduced motility but did not affect the cold shock protective action attributed to some factor in egg yolk by Mayer and Lasley (3). According to a survey conducted by the National Association of Artificial Breeders in 1950 (4), over half of the 48 associations replying indicated that they were using less than 50 per cent egg yolk. Several associations reported using concentrations as low as 14 to 16 per cent. Based on motility, Herrick (1) has suggested a pasteurized bovine semen diluter composed of three parts of homogenized yolk and five parts of citrate buffer solution.

As pointed out by Swanson (8), there are several advantages in using less yolk in preparing semen diluter, chief among which are the savings in time and expense which would be of particular value in large artificial breeding associations. Motility estimations can be made easier and with greater accuracy, since the spermatozoa are more distinct in appearance. Hurst (2) recently reported that use of less than 50 per cent egg yolk gave satisfactory fertility results in routine artificial breeding operations. Since no extensive field trials with varying concentrations of egg yolk in diluter have been reported, the present study was undertaken to determine the effect of using less than 50 per cent egg yolk upon the fertility of bovine semen.

EXPERIMENTAL PROCEDURE

By means of the split-sample technique, a field trial was conducted in cooperation with the NEPA Artificial Breeding Cooperative, Tunkhannock, Pa., from December 1, 1949, to January 14, 1950. Each of 108 ejaculates from 12 Holstein and six Guernsey bulls was divided into two portions. One portion was diluted with yolk-buffer containing 50 per cent egg yolk and served as the control, while the other was diluted with yolk-buffer containing either 25, 16.7 or 12.5 per cent egg yolk. These concentrations of egg yolk made comparisons possible between the standard 1:1 ratio of yolk to buffer and ratios of 1:3, 1:5 and 1:7 of yolk to buffer. The buffer solution contained 2.9 per cent sodium citrate dihydrate

Received for publication Feb. 16, 1951.

[1] Authorized for publication February 10, 1951 as paper no. 1653 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

as recommended by Salisbury *et al.* (7). Sulfanilamide was added at the rate of 600 mg. per 100 ml. of buffer solution.

The rate of semen dilution was the same for each bull, but varied between bulls. The average dilution rate was one part of semen to 46 parts of diluter, with a range of from 1 : 30 to 1 : 100.

The control and treated portions of diluted semen for each bull were shipped alternately to two similar groups of inseminators, so that all inseminators had equal opportunities to use semen with each type of diluter. Semen fertility was measured by the percentage of first-service cows not returning for further service within 6 mo. after the month in which they were inseminated. The per cent non-returns for each portion of each ejaculate was used for testing significance by "Students' " method (5).

RESULTS

The average fertility results involving 8,222 first-service cows are shown in table 1. A total of 4,064 cows were inseminated with control semen (50 per

TABLE 1

Average fertility of semen diluted with varying proportions of egg yolk and sodium citrate buffer based on 6-mo. non-returns to first-service cows

% Egg yolk in diluter	Ratio of egg yolk : citrate	No. services	% Non-returns
50	1 : 1	1,480	66.7
25	1 : 3	1,449	63.9
50	1 : 1	1,382	65.8
16.7	1 : 5	1,427	63.4
50	1 : 1	1,202	63.1
12.5	1 : 7	1,282	62.4

cent egg yolk) and 4,158 with treated semen (less than 50 per cent egg yolk). Statistical treatment revealed no significant differences in fertility when diluted semen containing 25, 16.7 or 12.5 per cent egg yolk was compared to diluted semen containing 50 per cent egg yolk. None of the differences between the means approached significance at the 5 per cent level of probability. Differences between results for the control semen and the semen diluted using less than 50 per cent egg yolk were greater for individual than for all bulls, but were inconsistent from one egg yolk concentration to another. In fact, variation among bulls was no greater than the experimental error. Thus, it appears that the amount of egg yolk in semen diluters can be reduced from 50 per cent to as low as 12.5 per cent without significantly affecting fertility.

Examination of the mean fertility data failed to reveal any trend toward lowered fertility with decreasing concentrations of egg yolk. In fact, the smallest mean difference (−0.7 per cent) was obtained at the lowest level of egg yolk tested (12.5 per cent). In 15 out of 36 ejaculates, the portion containing 25 per cent egg yolk resulted in a higher non-return rate than the portion containing 50 per cent egg yolk. Similar comparisons showed that fertility was higher in

18 out of 36 ejaculates at the 16.7 per cent level of egg yolk and in 20 out of 36 ejaculates at the 12.5 per cent concentration.

DISCUSSION

The results of this field trial indicate that it is possible to use a ratio of yolk to buffer in bovine diluters as wide as 1:7 and expect fertility results practically as good as those obtained with the 1:1 ratio originally recommended (6). If lowering the proportion of egg yolk exerts any effect upon fertility, from livability results (8), one might expect it to be adverse. The insignificant fertility differences obtained in the present experiment may have been influenced by the fact that practically all of the diluted semen was used for insemination within 72 hr. of age. Different results might be obtained with semen used for breeding purposes beyond this time, since Swanson (8) has shown that sperm motility is lower after the first 2 days of storage in yolk-buffer diluters containing 12.5 per cent egg yolk or less than in diluters with 50 per cent egg yolk. In addition, a critical concentration of egg yolk might have been reached if levels as low as 5 and 10 per cent had been tested.

The findings in the present paper are in agreement with the limited data recently presented by Hurst (2) in which 20 per cent egg yolk gave fertility results as satisfactory as 40 per cent egg yolk.

SUMMARY

Using the split-sample technique, comparisons were made between standard yolk-citrate diluter containing 50 per cent egg yolk (1:1, yolk to buffer) and diluters containing 25, 16.7 and 12.5 per cent egg yolk (1:3, 1:5 and 1:7, yolk to buffer). No significant differences in fertility were obtained in a field trial involving 8,222 first-service cows. Under present field conditions in which most semen is used for insemination purposes within 3 days, these data indicate that fertility results practically as good as those obtained with 50 per cent egg yolk can be expected with concentrations of egg yolk as low as 12.5 per cent.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance and cooperation of W. F. Schaefer, Jr., Manager, and of the laboratory staff of the NEPA Artificial Breeding Cooperative.

REFERENCES

(1) Herrick, J. B. The Effect of Homogenization, Pasteurization, and Lyophilization on Egg-Yolk Sodium Citrate Diluents for Bull Semen. Am. J. Vet. Research, 11: 159–160. 1950.

(2) Hurst, V. Results of Breeding Dairy Cows with Egg Yolk Citrate and Ortho Semen Diluters. J. Dairy Sci., 33: 395. 1950.

(3) Mayer, D. T., and Lasley, J. F. The Factor in Egg Yolk Affecting the Resistance, Storage Potentialities, and Fertilizing Capacity of Mammalian Spermatozoa. J. Animal Sci., 4: 261–269. 1945.

(4) National Association of Artificial Breeders. Semen Preparation and Preservation. Report at 3rd Ann. Meeting, Sept., 1950.

(5) PATERSON, D. D. *Statistical Technique in Agricultural Research.* 1st ed. McGraw-Hill Book Co., Inc., New York, N. Y. pp. 19–22. 1939.

(6) PHILLIPS, P. H., AND LARDY, H. A. A Yolk-buffer Pabulum for the Preservation of Bull Semen. J. Dairy Sci., **23**: 399–404. 1940.

(7) SALISBURY, G. W., KNODT, C. B., AND BRATTON, R. W. The Freezing Point Depression of Bull Semen and its Relation to the Diluter Problem. J. Animal Sci., **7**: 283–290. 1948.

(8) SWANSON, E. W. The Effect of Varying Proportions of Egg Yolk and Sodium Citrate Buffer in Bull Semen Diluters upon Sperm Motility. J. Dairy Sci., **32**: 345–352. 1949.

FLEXIBLE WRAPPERS FOR CHEDDAR CHEESE[1]

DARRELL D. DEANE[2] AND P. A. DOWNS
Dairy Husbandry Department, Agricultural Experiment Station,
University of Nebraska, Lincoln

The practice of marketing rindless natural cheddar cheese in a flexible wrapper rather than as a paraffined cheese is becoming more widespread. The advantages of this type of a consumer-unit package with the brand name printed on it have been demonstrated by the success of process cheese marketing.

As pointed out by Clunan (2), the cheese, in some instances, is cured in one of the flexible wrappers available or as a paraffined cheese and then at the desired age the old covering is removed, the cheese is cut into the desired size and rewrapped in the particular flexible wrapper used for the consumer-unit package. In other cases, the cheese is cut into the desired shape and size, wrapped at the time of manufacture, cured and sold with the original wrapper intact. These wrapping materials are transparent or semi-transparent, relatively impermeable to moisture vapor and they allow the escape of gases produced in the cheese.

Van Slyke and Price (9) have stated that one problem found in packaging natural cheddar cheese in flexible wrappers is the occurrence of visible mold growth on the surface of the cheese beneath the wrapper. These wrappers tend to prevent such mold growth if the cheese is packaged and pressed sufficiently, since they tend either to be sealed to the surface of the cheese or to cling tightly so that little or no oxygen is available for mold growth. Since the wrappers that are being used exclude oxygen from the cheese, they are effective in preventing mold growth as long as they are kept intact.

Studies have been made of the effect of certain chemicals that act as mold inhibitors upon the development of mold on the surface of cuts of cheddar cheese wrapped in moisture-proof wrappers. Ingle (5) ran several tests on fresh cut blocks of natural cheese 4 to 12 mo. of age dipped into propionate solutions before wrapping in tinfoil, moisture-proof cellophane or Pliofilm. His results indicated that the cheese dipped in 8 per cent propionic acid remained free of mold twice as long as the control cheese which had not been dipped. The use of 8 per cent calcium or sodium propionate was less effective than the 8 per cent propionic acid but somewhat better than the control. Sponging the wrappers instead of dipping the cheese had little effect in preventing mold growth. Miller (7) found that immersing cheddar cheese in 8 per cent propionic acid increased its mold-free life, when held at 60° F. in a moisture-proof covering, from the usual 3 to 5 days to 12 to 38 days. Solutions of 14 to 16 per cent calcium or sodium propionate were required for a similar effect. Miller found that the

Received for publication March 2, 1951.

[1] Published with the approval of the Director as Paper no. 524, Journal Series, Nebraska Agricultural Experiment Station.

[2] Present address: Dairy Industry Dept., Iowa State College, Ames.

results were influenced by the amount of contamination, the length of time that the cheese was immersed and the period of draining.

The purpose of this investigation was to study the effect of paraffin, aluminum foil and one of the flexible wrapping materials, Parakote, on the rate of ripening of natural cheddar cheese made from pasteurized milk and cured under various temperature conditions. It also was planned to observe the susceptibility of cheese wrapped with these materials to mold growth and loss of weight as compared to that found with paraffin-coated cheese. In this study, the use of aluminum foil was discontinued after the initial trials, since its use proved impractical because of its disintegration during ripening of the cheese.

Parakote is the registered trade name for a semi-transparent flexible cheese wrapper developed by the Menasha Products Company. The wrapper consists of two layers of cellophane bonded together with a thermoplastic laminant and is coated on the inner surface with a thermoplastic wax which adheres to the surface of the cheese after pressure and heat have been applied.

EXPERIMENTAL METHODS

The cheddar cheese used in this investigation was manufactured from milk obtained from a commercial source. The milk was standardized to 3.5 to 3.6 per cent milk fat, pasteurized at 143 to 145° F. for 30 min., cooled to a temperature of 88 to 90° F. and then transferred to the cheese vat. In the first series of trials, the cheese was made in 400-gal. cheese vats using approximately 2,000 lb. of milk, while in the second series of experiments 400 lb. of milk were placed in a 50-gal. cheese vat. In making all cheese, the method of Wilson (10) was followed.

In the first trial, which was comprised of two batches, the required number of loaves of cheese, each weighing approximately 5 lb., were taken from the press after pressing overnight and separated into three groups. One group was wrapped in Parakote, a second group was wrapped in uncoated aluminum foil 0.0015 in. thick and a third group was paraffined. Further subdivision of these groups was necessary in order that some of the cheese to be wrapped in either Parakote or aluminum could be dipped for a few seconds in a mold-inhibiting agent before the wrappers were applied. The mold-inhibiting materials used were a 12 per cent solution of Mycoban (a sodium propionate manufactured by Dupont) and a sodium hypochloride (B-K) made up to a strength of 400 ppm. Some cheese was wrapped without dipping. After wrapping with Parakote or aluminum foil, the cheese was pressed an additional 6 hr. The cheese in the third group was not treated with any mold inhibitor but was allowed to dry for 24 hr. at 65° F., stamped for identification and then dipped in paraffin at approximately 212° F. A sufficient number of loaves thus was prepared so that one set could be ripened at 50° F. and a duplicate set at 65° F. The relative humidity in the 65° F. curing room was approximately 65 per cent. Samples for moisture and fat determinations were taken before wrapping. At intervals of 5, 8, 12, 14 and 19 wk. all cheese in the first trial was removed from the curing room, weighed and then returned.

In the second series of trials, eight batches of cheese were made. Each batch consisted of six loaves of cheese, each weighing approximately 5 lb. The press cloths used to dress the cheese were soaked for 1.5 to 2 hr. in a sodium hypochlorite solution of 400 ppm. concentration at a temperature of 100 to 120° F. No other mold-inhibiting treatment was used. After pressing overnight each batch of cheese was divided into two groups of three loaves each. All three cheese in one group were cut into five pieces of approximately 1 lb. each. Each piece then was wrapped in Parakote, after which all five were assembled in the original loaf shape, wrapped in a sheet of wax paper to hold them together, then placed in a 5-lb. mold and pressed overnight. A composite representative sample was taken for chemical and moisture analysis from these loaves at the time of cutting and wrapping in the 1-lb. packages. The remaining three loaves of each batch were paraffined in the same manner as in the first trials. However, the cheese of this series was dipped a second time at 6 wk. of age. One cheese of each of the two lots was placed at 50° F., while the remaining two paraffined and two Parakote-wrapped cheese were placed at 65° F. One each of this latter group was removed after 12 wk. and placed at 50° F., and the remaining pair was left at 65° F. for the entire 24 wk.

All the cheese in the second trial was weighed and judged for flavor development by two judges at intervals of 6 wk. In sampling the Parakote cheese one of the individual packages was removed for scoring. In the case of the paraffined cheese approximately 1 lb. was cut off and the cut end of the remaining cheese was dipped to a depth of approximately 0.5 in. in paraffin at 212° F. All cheese sampled was weighed before and after sampling in order that the per cent of weight loss in each 6-wk. curing period could be calculated.

The chemical determinations made were carried out on samples taken at 1 day and 12 and 24 wk. of age. Total nitrogen was run by the Kjeldahl-Gunning-Arnold method (1) and the volatile fatty acids were determined by the method of Kosikowsky and Dahlberg (6). The analysis for water-soluble nitrogen was carried out according to the method of Sharp, as reported by Dahlberg and Kosikowsky (3). The moisture determinations were made when the cheese was 1 day old, by the method of Mojonnier and Troy (8).

EXPERIMENTAL RESULTS

The effect of the type of covering used and the temperature of curing on the rate of weight loss of the 5-lb. loaves of cheddar cheese is shown in tables 1 and 2. The moisture content of the two batches of cheese made in the first trial averaged 38.81 per cent when they were taken from the press. The loss of weight of those cheeses wrapped in Parakote was very small, regardless of the curing temperature used. The uncoated aluminum foil proved to be a very unsatisfactory wrapping material because after approximately 2 to 3 wk. at 65° F. or 4 wk. at 50° F., small holes appeared in the covering of all cheese wrapped in this material. The surface of the cheese exposed by these holes in the aluminum foil had an intensely metallic flavor. As the cheese aged, these holes became larger and in each instance the surface of the cheese exposed to the air soon showed mold

TABLE 1

Effect of type of covering and mold inhibitor on loss of weight and finish of 5 lb. loaves of cheddar cheese cured at 50° F.

Length of curing period	Average total weight lost						
	Parakote wrap			Aluminum foil wrap			Paraffin dip
	Mold inhibitor[a]			Mold inhibitor			Mold inhibitor
	A	B	C	A	B	C	A
(wk.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
5	0.05	0.04	0	0.07	0.20	0	2.11
8	0.05	0.09	0	0.65	1.02	0.05	3.23
12	0.05	0.09	0	1.69	2.28	1.58	4.57
14	0.05	0.14	0	2.39	3.16	2.18	5.59
19	0.05	0.14	0	4.60	5.88	3.96	7.91
No. of cheese	3	4	3	3	4	2	5
Description of mold growth (19th week)	Slight; along folds	None	Trace on end folds	Abundant mold growth on exposed surface and under part of remaining foil			Very little

[a] Mold inhibitor used: A = no treatment; B = sodium propionate dip (12%); C = sodium hypochlorite dip (400 ppm.).

growth. The cheese cured in aluminum foil at 65° F. was discarded after 12 wk. of curing because most of the wrapper had disintegrated. Until these holes appeared, however, the weight loss was very small. The paraffined cheese lost the most weight of the cheese cured at 50° F. This group lost more weight than

TABLE 2

Effect of type of covering and mold inhibitor on loss of weight and finish of 5-lb. loaves of cheddar cheese cured at 65° F.

Length of curing period	Average total weight lost						
	Parakote wrap			Aluminum foil wrap			Paraffin dip
	Mold inhibitor[a]			Mold inhibitor			Mold inhibitor
	A	B	C	A	B	C	A
(wk.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
5	0.00	0.05	0	0.55	0.72	0.74	2.66
8	0.06	0.09	0	2.36	2.68	3.00	4.74
12	0.00	0.14	0	3.48	4.65	4.64	4.74
14	0.06	0.14	0.14	[b]	[b]	[b]	5.47
19	0.06	0.14	0.14				5.43
No. of cheese	3	4	4	3	3	3	5
Description of mold growth (19th week)	Trace at folds	None	Trace at folds	Abundant mold growth on exposed surface and under part of remaining foil			Slight

[a] Mold inhibitor used: A = no treatment; B = sodium propionate dip (12%); C = sodium hypochlorite dip (400 ppm.).

[b] Cheese discarded.

the cheese cured in aluminum foil even though holes did develop in the metallic wrapper. When the cheese was held at 65° F., the paraffin-coated cheese again lost the most weight. In the cheese wrapped in aluminum foil the amount of weight lost was greatest when the loaves were cured at 65° F., probably because of the more rapid disintegration of the covering at this temperature. Parakote-wrapped cheese lost slightly more weight when held at 65° F. as compared with 50° F., but the difference was not great.

The effect of the mold inhibiting treatments used also is shown in tables 1 and 2. In the cheese wrapped in aluminum foil, the treatment used had no appreciable effect upon the rate of mold growth on the surface of the cheese exposed when the holes appeared in the covering. No appreciable mold growth was found in the cheese wrapped in Parakote, as long as the wrapper was intact. Slight

TABLE 3

Mean values of per cent of original weight lost and of rate of flavor development of paraffined and Parakote-wrapped cheddar cheese cured under different temperature conditions[a]

Age of cheese	Cured at 65° F. for 24 wk.				Cured at 65° F. for 12 wk. then at 50° F. for 12wk.				Cured at 50° F. for 24 wk.			
	Paraffin		Parakote		Paraffin		Parakote		Paraffin		Parakote	
	Weight loss	Flavor development[b]	Weight loss	Flavor development	Weight loss	Flavor development	Weight loss	Flavor development	Weight loss	Flavor development	Weight loss	Flavor development
(*wk.*)	(%)		(%)		(%)		(%)		(%)		(%)	
6	1.69	2.0	0.10	2.0	1.81	2.0	0.11	2.0	0.63	0	0.12	0
12	2.85	3.7	0.18	3.4	3.08	3.7	0.17	3.4	1.57	1.2	0.18	0.8
18	4.65	6.1	0.27	5.6	4.42	4.6	0.26	4.0	2.88	2.2	0.25	1.8
24	6.32	7.8	0.35	7.6	6.03	5.3	0.31	4.7	4.09	2.9	0.33	2.4

[a] Each figure given is the mean of 8 cheeses. The pooled standard deviations of these means are 0.31 for per cent weight loss and 0.22 for flavor development.

[b] Intensity scale for flavor development:

0 = No development
0.5–2.0 = Slight development
2.5–4.0 = Moderate development
4.5–6.0 = Definite development
6.5–7.0 = Very definite
7.5–8.0 = Pronounced
8.5–9.0 = Very pronounced

mold growth was observed along the folded seam of the wrapper on most of the cheese not dipped or on that dipped in the sodium hypochlorite solution. No mold growth was found on the Parakote-wrapped cheese dipped in the 12 per cent sodium propionate solution previous to wrapping. The cheese paraffined without dipping showed little mold growth when cured at 50° F.; however, slight mold growth was found when the cheese was cured at 65° F.

The data obtained in the second series of trials comparing weight loss and flavor development of the cheese wrapped in Parakote with that of the paraffin-dipped cheese are presented in table 3. The average moisture content of the eight batches of cheese was 38.51 per cent. It is evident that again the loss of weight of cheese wrapped in Parakote was less than that of the paraffin-dipped cheese. An analysis of variance reveals highly significant interactions between

age of cheese and treatment, as well as between storage temperature and treatment. A clearer picture of the relative importance of age, storage temperature and treatment (Parakote-wrapped *versus* paraffined) is arrived at by computing the components of variance which are given in table 4 for both weight loss and

TABLE 4

Components of variance in per cent weight loss and flavor development in cheddar cheese cured under different temperature conditions and treated in two ways via either paraffined or wrapped in Parakote

Source of variance	Weight loss		Flavor development	
	Variance	Per cent of total variance	Variance	Per cent of total variance
Treatment	4.37	61.5	0.05	0.8
Age	0.19	2.6	2.49	39.6
Temperature	0.01	0.1	2.80	44.5
Temperature × treatment	0.37	5.3	0	0
Age × treatment	1.38	19.4	0.01	0.1
Age × temperature	0	0	0.56	8.9
Error	0.79	11.1	0.39	6.1
Total	7.11	100.0	6.30	100.0

flavor development. Nearly 62 per cent of the variance can be ascribed to treatment and an additional 19 per cent to the combined effect of age and treatment. Thus, 81 per cent of the total variance in weight loss is due to the effects of treatment and age. The weight loss of the Parakote-wrapped cheese in this series of trials was greater than that reported in the first trials (tables 1 and 2). This probably is because in the first trial the cheese was wrapped as a single 5-lb. loaf, while in the second the loaf was made up of five 1-lb. portions, individually wrapped, with more surface exposure per pound of cheese. The per cent of weight lost by the paraffined cheese in these latter trials was less than that lost in the first series of experiments (tables 1 and 2). One factor which probably reduced the loss of weight in this second series was the second paraffin dip the cheese received at 6 wk. of age.

In relatively few instances was mold growth observed on the surface of the 1-lb. cuts of cheese in the wrapper as long as the covering remained intact. This reduced mold growth may be explained by the fact that, although the cheese was not dipped in a mold-inhibiting solution, it was pressed overnight after wrapping. This caused the Parakote to adhere closely to the surface of the cheese and definitely limited mold growth. Occasionally, a small spot of mold could be found on one of the end seams but it was infrequent. When mold growth was encountered, it was found more frequently on the cheese cured at 65° F. than at 50° F.

The influence of the Parakote wrapper on the flavor development of the cheese also is shown in table 3. Age and temperature here are the two main factors influencing flavor development. The analysis of variance showed highly significant differences due to age and temperature. The development of flavor was somewhat more rapid in the paraffined cheese than in the Parakote-wrapped

cheese under the same conditions of curing and the difference is just above the 5 per cent point of significance. The relative importance of age, storage temperature and treatment on flavor development may be seen from table 4. Forty-four per cent of the variance is due to differences in storage temperature and 40 per cent is due to the differences in age. Nine per cent of the variance is due to interaction between age and temperature and all together 93 per cent of the variance in flavor development is due to age, storage temperature and the combined effect of these two factors. Whether the cheese is dipped in paraffin or wrapped in Parakote is of no importance on flavor development as compared with the other two sources of variance. It was observed that the flavors developed in the cheese in these two coverings were not always similar. The cheese cured in Parakote was characterized in some instances by a slight volatile acid aroma and flavor and did not develop the "nutty" flavor characteristic of the paraffined cheese. The intensity of the volatile acid flavor was directly proportional to the length of time the cheese was held at 65° F.

TABLE 5

Effect of covering and temperature of curing on the total nitrogen, water-soluble nitrogen and volatile fatty acid content of cheddar cheese made from pasteurized milk[a]

Cheese		Total nitrogen (%)		Water-soluble nitrogen (as % of cheese)		Water-soluble nitrogen (as % of total nitrogen)		Volatile fatty acids (ml. 0.1*N* Na OH/100 g.)	
Age	Covering	50° F.	65° F.	50° F.	65° F.	50° F.	65° F.	50° F.	65° F.
1 d.	Paraffin	3.90	3.90	0.29	0.29	7.44	7.44	7.69	7.69
	Parakote	3.90	3.90	0.29	0.29	7.44	7.44	7.69	7.69
12 wk.	Paraffin	3.98	4.06	0.91	1.33	22.86	32.76	16.60	20.86
	Parakote	3.94	3.92	0.93	1.30	23.60	33.16	14.54	22.78
24 wk.	Paraffin	4.07	4.24	1.28	1.80	31.45	42.45	20.32	26.75
	Parakote	3.98	3.96	1.27	1.70	31.91	42.93	18.82	30.23

[a] Each figure is average of 8 cheese in duplicate.

In table 5 are presented data showing the effect of type of covering and temperature of curing on the total and soluble nitrogen fractions, as well as the volatile fatty acids. The samples of cheese taken at 1 day of age were composites and were obtained before the covering was applied, which explains why those figures are the same for both covering and temperature involved. The increase in total nitrogen found as the cheese aged was due to the loss of moisture. The water-soluble nitrogen definitely increased with the increase in temperature of curing and length of ripening period. At 12 and 24 wk. of age the water-soluble nitrogen in the majority of cases was slightly higher in the paraffined cheese than in those wrapped in Parakote, but the differences were not appreciable.

The results obtained in determining the volatile fatty acids indicate that increasing the temperature of ripening and length of curing period caused a very definite increase in this fraction. The type of covering made no significant difference. Thus, the characteristic slight volatile acid flavor and aroma of the

Parakote-wrapped cheese does not have any significant relationship to the values for volatile fatty acids found by chemical analysis. It should be mentioned, however, that the slight volatile acid flavor and aroma of the Parakote-wrapped cheese was more noticeable when the cheese was cured at 65 instead of 50° F.

The data obtained in this investigation indicate that by the use of the Parakote wrapper for the curing of natural cheddar cheese, the loss in weight was reduced to a very low percentage, even after curing at a temperature of 65° F. for 6 mo. When the cheese was paraffined, however, an appreciable per cent of original weight was lost. Hansen (4) previously reported that cheese lost 4.81 per cent of its weight after 20 wk. and 6.41 per cent after 24 wk. of curing at 50° F. At 60° F., for the same lengths of time, Hansen found the losses were 7.22 and 8.71 per cent, respectively. The data reported in this study show losses less than this. The loss in weight found in the second series of trials (table 3) indicates that paraffining the cheese a second time at 6 wk. of age reduced, to some extent, loss of moisture.

It also was found that the rate of flavor development was slightly slower when the cheese was cured in the Parakote wrapper and the cheese did not have the characteristic "nutty" flavor found in the paraffined cheese. The ripening of both paraffined and Parakote-covered cheese was accelerated by holding the cheese at 65° F. No consistent off-flavor was found that could be attributed to the use of a Parakote wrapper other than a flavor best described by the term "slight volatile acid flavor." It was found that the surface mold growth could be held to a minimum if the press cloths were soaked in a strong sodium hypochlorite solution of approximately 400 ppm. for 1.5 to 2 hr. before applying and the cheese was pressed overnight after wrapping in the Parakote. If a break or a tear occurred in the wrapper or the folds were not sealed properly, mold growth did appear. Increasing the curing temperature from 50° F. to 65° F. brought about a definite increase in the soluble protein and total volatile fatty acid fraction.

SUMMARY

Use of a Parakote wrapper reduced the weight loss of cheddar cheese during curing to a very small amount, as compared with that found when the cheese was paraffined.

The per cent of weight lost by the Parakote-wrapped cheese was less when the 5-lb. loaf was wrapped intact rather than as five 1-lb. portions.

Only slight, if any, mold growth was found on the surface of the cheese wrapped in Parakote and pressed overnight, as long as the wrapper remained intact.

Uncoated aluminum foil was unsatisfactory as a cheese wrapper since it disintegrated during curing and mold growth occurred on the exposed surface of the cheese.

The 12 per cent sodium propionate solution was satisfactory as a mold inhibitor. Sodium hypochlorite in the concentration used (400 ppm.) was not as effective.

The use of a Parakote wrapper resulted in a cheese which ripened slightly slower than the corresponding paraffined cheese, regardless of whether the curing temperature was 50 or 65° F., but this effect of the type of cover was of little importance compared with the effect of age and temperature on flavor development.

The flavor produced in the cheese wrapped in Parakote was slightly different from that of the paraffined cheese in that the flavor, developed toward the end of the ripening period in some of the Parakote-wrapped cheese, was described as a "slight volatile acid flavor," while that of the paraffined cheese was called "nutty."

ACKNOWLEDGMENT

The authors wish to acknowledge the help of M. Plum who did the statistical analysis of the data presented in this paper.

REFERENCES

(1) Association of Official and Agricultural Chemists. *Official and Tentative Method of Analysis.* 6th ed. Washington, D. C. 1945.
(2) Clunan, A. B. Consumer-size Packages Revolutionizing Cheese Industry. Natl. Butter and Cheese J., **40** (9): 24–25. 1949.
(3) Dahlberg, A. C., and Kosikowsky, F. V. The Flavor, Volatile Acidity and Soluble Protein of Cheddar and Other Cheese. J. Dairy Sci., **30**: 165–174. 1947.
(4) Hansen, H. C. The Influence of Temperature on the Curing of Cheddar Cheese. Can. Dairy Ice Cream J., **25** (6): 48, 50. 1946.
(5) Ingle, J. D. Some Preliminary Observations on the Effectiveness of Propionates as Mold Inhibitors on Dairy Products. J. Dairy Sci., **23**: 509. 1940.
(6) Kosikowsky, F. V., and Dahlberg, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acid of Cheese. J. Dairy Sci., **29**: 861–871. 1946.
(7) Miller, F. W., Jr. Mold Growth on Cheddar Cheese and its Control. Proc. of First Annual Conference of Institute of Food Technologists. Pp. 153–158. 1940.
(8) Mojonnier, T., and Troy, H. C. *Technical Control of Dairy Products.* 1st ed. Mojonnier Bros. Co., Chicago, Ill. 1922.
(9) Van Slyke, L., and Price, W. V. *Cheese.* Rev. ed. Orange Judd Pub. Co., New York. Pp. 345–350. 1949.
(10) Wilson, H. L. Making American Cheddar Cheese of Uniformly Good Quality. U.S.D.A. Mimeo. BDIM-947. 1942.

PRELIMINARY STUDIES OF THE EFFECT OF ACIDO-PROTEOLYTIC ORGANISMS AND TEMPERATURES OF CURING ON THE RIPENING OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK[1]

DARRELL D. DEANE[2]

Dairy Husbandry Department, University of Nebraska, Lincoln

The use of pasteurized milk in the manufacture of cheddar cheese has not been universally practiced even though it possesses certain advantages from the standpoint of yield and quality of the cheese obtained. Development of flavor, however, in cheddar cheese made from pasteurized milk is slower and the flavor is less pronounced than that of cheese made from raw milk.

One approach that has been made in an effort to accelerate the ripening process in pasteurized milk cheddar cheese has been through the use of pure cultures of selected organisms added with or in substitution for the usual commercial cheese starter. The organisms that have been studied include those termed "acido-proteolytic." This designation has been given those organisms that ferment the lactose fraction of milk and hydrolyze casein in the resultant acid medium. The importance of the role of these organisms in the ripening of cheese has been pointed out by Gorini (4). Hucker and Marquardt (8) found, however, that the use of an acido-proteolytic coccus as described by Gorini (4) produced a decidedly bitter flavor in the cheddar cheese made from pasteurized milk. On the other hand, Deane (3) reported that an acido-proteolytic coccus, when used to supplement the commercial cheese starter in making cheddar cheese from raw milk, did improve the flavor development. Lane (10) found that small amounts of *Streptococcus liquefaciens* used in making cheddar cheese from pasteurized milk produced a well-ripened cheese in a comparatively short time, while a relatively large number of these organisms produced a bitter flavor and soft bodied cheese. Hansen (5) reported that the use of *S. liquefaciens* improved the flavor but did not materially influence the nitrogenous decomposition of cheddar cheese made from pasteurized milk. Wojtkiewicz and Inikkoff (17) added small amounts (0.4 per cent) of selected proteolytic, strongly acidifying cultures of *Streptococcus lactis, Lactobacillus bulgaricus* and *Lactobacillus casei* to cheese milk and found that these cultures increased the rate of flavor production in cheddar cheese.

The effect of temperature on the rate of ripening also has been studied by other workers. The earlier work regarding this phase of the study was reviewed by Hanson *et al.* (7). These investigators found that the curing temperature for cheddar cheese made from pasteurized milk could be increased to a maximum of 70° F. with desirable results. Curing at 80° F., however, resulted in an in-

Received for publication March 2, 1951.

[1] Published with the approval of the Director as Paper no. 530, Journal Series, Nebraska Agricultural Experiment Station.

[2] Present address: Dairy Industry Dept., Iowa State College, Ames.

ferior cheese in most cases. The higher temperatures increased the rate of flavor development and greatly decreased the ripening time, but in many cases high rates of protein breakdown resulted, causing the cheese to receive lower flavor scores. Wilson *et al.* (16) reported that cheddar cheese made from high quality pasteurized milk could be cured at 50° F. or higher, while Sanders *et al.* (13) found that pasteurized milk cheese cured at 60° F. ripened faster and developed more flavor than the control cheese held at 50° F. A curing temperature of 70° F. was reported as being too high. Hansen (6) reported that at 60° F. more flavor was developed in cheddar cheese in 3 mo. than in 6 mo. at 40 to 50° F. Lochry (11) recently reported that cheese made from pasteurized milk of good quality was ripened as fully in 3 to 4 mo. at 60° F. as that cured for 6 mo. at 50° F. The cheese cured at 60° F. developed not only more flavor but also a better flavor than that cured at 50° F.

This study was carried out in an effort to obtain further information concerning the use of acido-proteolytic organisms and varying curing temperatures upon the ripening of cheddar cheese made from pasteurized milk.

EXPERIMENTAL METHODS

The organisms used in this investigation were all acido-proteolytic in nature. One was a culture of *S. liquefaciens* obtained from Iowa State College. A second, designated as A. P. no. 1 was an unidentified micrococcus isolated from raw milk. This organism grew at 10 and 45° C., produced ammonia from peptone and acid from glycerol and mannitol, but not raffinose. Nutrient gelatin stabs were completely liquefied in 24 hr. at 32° C. Saccate liquefaction was observed in 48 hr. at 20° C. Nitrites were not produced from nitrates. At 32° C. litmus milk showed acid coagulation in 24 hr. followed by peptonization with reduction after 48 to 72 hr. The reaction of the litmus milk after incubating 7 days at 32° C. was pH 4.3 to 4.4. This micrococcus varied in size from 0.5 to 0.8 μ in diameter.

A third organism, designated as A. P. 5A, also was an acido-proteolytic micrococcus isolated from a 4-yr.-old cheddar cheese. This organism was the least proteolytic of the three cultures used in this study. Litmus milk showed acid coagulation and slight proteolysis after 7 days at 32° C. with a pH of 5.1 to 5.2. Nitrates were not reduced to nitrites. Ammonia was produced from 4 per cent peptone medium and acid from glycerol but not from mannitol or raffinose. Nutrient gelatin showed saccate liquefaction after 1 wk. at 20° C. The organism varied in diameter from 0.6 to 1.0 μ.

The milk used to prepare the cheese was obtained from a commercial milk supply, standardized with skimmilk to a fat content of 3.5 to 3.6 per cent and then pasteurized at 143 to 145° F. for 30 min. The milk then was cooled to 88 to 90° F., divided into two lots of 400 lb. each and poured into small cheese vats. The cheese was made according to the method of Wilson (15), keeping the manufacturing process as uniform as possible and using 4.5 hr. from setting to milling. The control cheese was made in one vat with 0.75 per cent of a commercial cheese culture, while the experimental cheese was made in the second vat with the same

amount of commercial starter plus a pure culture of the organism being studied. A 0.5 per cent inoculum of the culture was used to prepare all experimental cheese with one exception, in which 0.10 per cent of *S. liquefaciens* was used in addition to the commercial starter.

Six loaves of cheese, each weighing approximately 5 lb., were selected from each vat of milk. A composite sample for analysis was taken from three of these loaves. The remaining three were stamped with the date of manufacture and identification number and used in this study. After drying for 24 hr. at a temperature of 65° F., the cheese was paraffined at approximately 210° F. One of the three loaves of each batch was placed at 50° F., while the remaining two were placed at 65° F. One of these latter two remained at 65° F. for 12 wk. before being transferred to the 50° F. room, while the other was cured entirely at 65° F. The relative humidity of the 65° F. room was maintained at approximately 65 per cent.

Two judges examined the cheese at intervals of 6 wk. to evaluate the amount of flavor development and any changes in body and texture that occurred during the ripening process. Any defects observed were noted.

Chemical analyses were made on the cheese when 1 day old and at 12 and 24 wk. of age. Total nitrogen was run by the Kjeldahl-Gunning-Arnold method (1), while the volatile fatty acids were determined by a method proposed by Kosikowsky and Dahlberg (9). Soluble nitrogen determinations were made according to the method of Sharp, as reported by Dahlberg and Kosikowsky (2). The total solids determinations, made on the cheese when 1 day old, were made by the Mojonnier procedure (12). The per cent of milk fat was determined by the modified Babcock test (14).

EXPERIMENTAL RESULTS

The results obtained relative to the acidity development during the manufacture of the cheddar cheese made from pasteurized milk, as well as its composition, are presented in table 1. Addition of 0.5 per cent of the acido-proteolytic micrococcus A.P. 5A or A.P. no. 1 to the cheese milk had little or no effect upon the rate of acid development in the cheese during its manufacture. However, when *S. liquefaciens* was used the acidity developed more slowly during cheddaring and the final moisture was higher than that found in the control cheese. It also was observed that the titrable acidity produced by *S. liquefaciens* in the sterile milk used to make the starter was greater than that produced by either of the two acido-proteolytic micrococci under the same conditions. Nevertheless, the acidity at the time of milling was less in the cheese made with *S. liquefaciens* than in any of the other cheese made.

The effect of these cultures on the rate and degree of flavor development in the experimental cheese is shown in table 2. The two acido-proteolytic micrococci (A.P. 5A and A.P. no. 1) had no pronounced effect upon the rate of flavor development or its intensity. Many times, however, the experimental cheese had a slightly more advanced flavor than the control cheese. Since these differences in flavor were slight, they were not thought to be significant. No consistent off-

flavors were found in the experimental cheese made with these two cultures of acido-proteolytic micrococci, even when the cheese was cured at 65° F.

When *S. liquefaciens* was used to prepare the experimental cheese a distinct effect upon both flavor and rate of flavor development was found. In the experimental cheese made with 0.10 per cent *S. liquefaciens* and held at 65° F., a more advanced development was found after 6 wk. of curing than was found in the control cheese cured at 65° F. for 18 wk. By the end of the 12th week of curing at 65° F., however, the experimental cheese were too bitter to be edible. When cured entirely at 50° F., the cheese made with 0.10 per cent *S. liquefaciens* also showed an accelerated development of flavor, but again the cheese became too bitter to be edible between the 12th and 18th week of curing. When greater

TABLE 1

The rate of acid development during the manufacture of pasteurized milk cheddar cheese and the composition of the cheese made with commercial lactic cultures alone and in combination with certain pure cultures

Manufacturing data	Control	Acido-proteolytic cultures used			
		A.P. #1	A.P. 5A	*S. liquef.*,	*S. liquef.*
No. of trials	8	3	3	1	1
Fresh milk, titr. acid (%)	0.174	0.178	0.170	0.180	0.170
Commercial starter (%)	0.750	0.750	0.750	0.750	0.750
Titr. acid of com. starter (%)	0.840	0.857	0.827	0.830	0.840
Experimental culture (%)	.....	0.500	0.500	0.100	0.500
Titr. acid of exp. culture (%)	.....	0.373	0.390	0.860	1.100
Setting acidity (%)	0.184	0.190	0.183	0.185	0.180
Cutting acidity (%)	0.114	0.115	0.115	0.115	0.115
Steam off, acidity (%)	0.122	0.123	0.122	0.125	0.120
Dipping acidity (%)	0.133	0.135	0.135	0.135	0.135
Milling acidity (%)	0.495	0.493	0.497	0.390	0.430
Cheese out of Press					
Yield/cwt. milk (*lb.*)	10.58	10.61	10.63	10.76	10.48
Moisture (%)	38.59	38.49	38.77	39.43	41.15
Fat (%)	30.72	30.75	30.75	30.75	30.25
Protein (%)	24.87	24.93	25.03	24.04	22.90

amounts of *S. liquefaciens* were added to the milk, the resulting cheese developed an undesirable, soft, pasty body and exuded a fluid with a very bitter flavor. This fluid, or serum, first was noticed at approximately the same time that these cheese developed their characteristic bitter flavor.

Table 2 also shows the effect of curing temperature on the rate of flavor development. The rate of flavor development in both the control and the experimental cheese definitely was accelerated by increasing the curing temperature from 50 to 65° F. A moderate development of the aged cheddar cheese flavor was developed by the 12th week of curing at 65° F. However, when the cheese was held at 50° F. it required approximately 24 wk., or twice as long, to develop a flavor of equal intensity. The practice of placing the cheese at 50° F. after curing for the first 12 wk. at 65° F. resulted in the slower rate of ripening from that point on as compared to the rate of ripening during the first 12 wk. There were no defi-

nite off-flavors found that could be correlated with the use of 65° F., as compared with 50° F., as a curing temperature.

Table 3 presents the data obtained in analyzing the cheese held at the various curing temperatures for total and water-soluble nitrogen, as well as the volatile fatty acid content. These cheese were held at the indicated temperature for the entire period of 24 wk. It is apparent from these data that neither of the acido-proteolytic micrococci, 5A or no. 1, had any consistent effect on the water-soluble nitrogen or volatile fatty acids of the cheese. On the other hand, the culture *S. liquefaciens* did increase appreciably both the per cent of water-soluble nitrogen and the volatile fatty acid content over that found in the controls. The cheese

TABLE 2

The effect of pure cultures of acido-proteolytic organisms and the curing temperature upon the rate of flavor development in pasteurized milk cheddar cheese

Cheese	No. of trials	Intensity of flavor development of the cheese[a]											
		Cured at 65° F. entirely				Cured at 65° F. 12 wk. then 50° F. 12 wk.				Cured at 50° F. entirely			
		Weeks of age				Weeks of age				Weeks of age			
		6	12	18	24	6	12	18	24	6	12	18	24
Control	3	1.8	3.8	6.2	7.8	1.8	3.8	4.8	5.3	0	1.8	2.8	3.3
" + 0.5% A.P. 5A	3	2.5	4.7	6.3	8.2	2.5	4.7	5.3	5.0	0	2.0	3.0	3.0
Control	3	2.0	3.5	5.8	7.5	2.0	3.5	4.2	5.2	0	1.0	1.8	2.2
" + 0.5% A.P. #1	3	2.0	3.7	6.7	8.0	2.0	3.7	4.3	5.2	0	0.8	1.8	2.7
Control	1	2.0	3.5	7.0	8.0	2.0	3.5	5.0	5.0	0	1.0	2.0	4.0
" + 0.1% *S. liquef.*	1	5.5	7.0	[b]	[b]	5.5	7.0	[b]	[b]	0	2.0	[b]	[b]
Control	1	2.5	4.0	6.0	8.0	2.5	4.0	5.0	6.0	0	0.0	2.0	3.0
" + 0.5% *S. liquef.*	1	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]

[a] Intensity scale for flavor development:
0 = No development
0.5–2 = Slight development
2.5–4 = Moderate development
4.5–6 = Definite development
6.5–7 = Very definite
7.5–8 = Pronounced
8.5–9 = Very pronounced

[b] Too bitter to be palatable.

made with 0.10 per cent of *S. liquefaciens* had a higher volatile fatty acid content than the cheese made with 0.5 per cent *S. liquefaciens;* however, the use of 0.5 per cent *S. liquefaciens* resulted in a greater production of water-soluble nitrogen than was found with 0.10 per cent of this same organism. The results of the analysis for volatile fatty acids also were somewhat inconsistent since considerable variation in the data was found when analyzing control cheese made under very similar conditions. These differences became more marked as the cheese aged. The results obtained when the control cheese were analyzed for water-soluble nitrogen were much more in agreement.

A ripening temperature of 65° F. increased the amount of both water-soluble nitrogen and volatile fatty acids found, as compared with that found in the cheese cured at 50° F. There seemed to be no relationship between the rate of increase of water-soluble nitrogen and the increase found in the volatile fatty acid content.

TABLE 3

The effect of pure cultures of acido-proteolytic organisms and curing temperature on the total and water-soluble nitrogen and volatile fatty acids of cheddar cheese made from pasteurized milk

	No. trials	Total nitrogen		Water-soluble nitrogen		Volatile fatty acids	
		50° F.	65° F.	50° F.	65° F.	50° F.	65° F.
		(%)	(%)	(%)	(%)	(*ml.* 0.1 *N* NaOH/100 *g.*)	
				1 d. old			
Control	3	3.91	3.91	0.27	0.27	8.41	8.41
" + A.P. 5A	3	3.92	3.92	0.26	0.26	7.69	7.69
Control	3	3.89	3.89	0.28	0.28	6.40	6.40
" + A.P. #1	3	3.91	3.91	0.27	0.27	6.98	6.98
Control	1	3.90	3.90	0.28	0.28	9.82	9.82
" + 0.1% *S. liquef.*	1	3.77	3.77	0.34	0.34	13.34	13.34
Control	1	3.88	3.88	0.39	0.39	7.23	7.23
" + 0.5% *S. liquef.*	1	3.59	3.59	1.17	1.17	21.41	21.41
				12 wk. old			
Control	3	3.98	4.03	0.87	1.29	17.55	25.35
" + A.P. 5A	3	4.00	4.03	0.91	1.34	19.01	21.43
Control	3	4.00	4.09	0.91	1.34	15.91	16.77
" + A.P. #1	3	4.01	4.04	0.96	1.40	17.51	21.57
Control	1	3.99	4.14	1.03	1.40	19.29	25.47
" + 0.1% *S. liquef.*	1	4.05	4.02	1.76	2.24	23.77	30.89
Control	1	3.88	3.97	0.94	1.39	13.14	15.08
" + 0.5% *S. liquef.*	1	3.66	3.79	2.95	2.88	25.08	28.85
				24 wk. old			
Control	3	4.08	4.23	1.28	1.73	19.98	24.42
" + A.P. 5A	3	4.14	4.21	1.02	1.81	19.26	26.22
Control	3	4.11	4.26	1.33	1.89	17.68	25.08
" + A.P. #1	3	4.14	4.21	1.32	1.84	20.86	25.61
Control	1	4.06	4.28	1.24	1.74	29.02	35.60
" + 0.1% *S. liquef.*	1	4.01	4.17	2.38	2.61	32.31	48.04
Control	1	3.97	4.14	1.18	1.87	20.56	29.92
" + 0.5% *S. liquef.*	1	3.77	4.10	3.13	3.20	28.22	32.67

DISCUSSION OF RESULTS

When pure cultures of acido-proteolytic organisms were added to the cheese milk in addition to the usual commercial lactic cultures, *S. liquefaciens* definitely increased the development of the flavor in the cheese but its use proved unsatisfactory because the cheese developed an intense bitter flavor in a relatively short time and the body became soft and pasty. The other two acido-proteolytic micrococci did not produce this intense bitter flavor or soft body nor did they have any appreciable influence upon either the rate of flavor development or the protein breakdown in the cheese. Culture A.P. no. 1 was more proteolytic in nature than culture A.P. 5A when grown in milk, yet it did not seem to increase the rate of protein breakdown over that produced when the latter culture was used. These acido-proteolytic micrococci showed much less proteolytic action in milk than *S. liquefaciens*. When the data obtained in this investigation are considered

in connection with those reported by Hucker and Marquardt (8), Lane (10), Hansen (5)and Deane (3), it seems that if acido-proteolytic organisms are to be used they will have to be studied on a strain basis.

The second phase of this investigation substantiated the work of other investigators in that the curing room temperature was shown to have a very definite effect upon the rate of ripening. While a temperature of 65° F., definitely shortened the ripening time as compared with 50° F., no definite off-flavors were observed that could be attributed to this higher temperature. However, cheese cured at 65° F. with a relative humidity of approximately 65 per cent did require more attention than cheese cured at 50° F., if mold growth and the presence of cheese mites were to be held to a minimum. If it is possible to avoid mold growth and mite infestations, curing temperatures of 60 to 65° F. might well be considered, since the time required for flavor development is greatly reduced with a resulting decrease in curing costs.

SUMMARY

The use of *S. liquefaciens* was unsatisfactory as a means of increasing the rate of flavor development in cheddar cheese made from pasteurized milk.

The addition of *S. liquefaciens* did increase the amount of water-soluble nitrogen and volatile acids. However, the cheese made with as little as 0.10 per cent of this culture developed a flavor so bitter that it became unpalatable.

Two acido-proteolytic micrococci, when added to the cheese milk in amounts of 0.50 per cent, did not significantly increase the rate of production of water-soluble nitrogen or volatile fatty acids during the 24-wk. curing period. These cultures were less proteolytic in milk than *S. liquefaciens* and the cheese did not develop the bitter flavor that was produced with the latter organism.

Curing cheddar cheese at 65° F. increased the rate of flavor development as well as the total and water-soluble nitrogen and volatile fatty acids as compared with 50° F. Cheese held at 65° F. for 24 wk. had a definite-to-pronounced flavor development with no off-flavor attributed to this higher temperature. Cheese ripened at 50° F. for the same period had a slight to moderate flavor development.

ACKNOWLEDGMENT

The author is indebted to H. P. Davis for his help in planning the study, to L. K. Crowe for his advice in respect to the analytical work and to P. A. Downs for his help in judging the various cheese. The assistance given by Glen Claybaugh and James Mickle when making the chemical analyses also is greatly appreciated.

REFERENCES

(1) Association of Official and Agricultural Chemists. *Official and Tentative Methods of Analysis.* 6th ed. Washington, D. C. 1945.

(2) Dahlberg, A. C., and Kosikowsky, F. V. The Flavor, Volatile Acidity and Soluble Protein of Cheddar and Other Cheese. J. Dairy Sci., 30: 165–174. 1947.

(3) Deane, D. D. Bacteriological Observations on the Ripening of Cheddar Cheese. Ph.D. Thesis. Pennsylvania State College. 1942.

(4) GORINI, C. Some New Contributions Regarding the Significance of the Acidoproteolytes in Cheese Ripening. Tenth World's Dairy Congress, Rome-Milan. Section II. pp. 23–32. 1934.

(5) HANSEN, H. C. The Influence of Certain Bacteria on the Ripening of Cheddar Cheese Made from Pasteurized Milk. B. W. Hammer Panegyric, pp. 123–132. Collegiate Press, Inc., Ames, Iowa. 1937.

(6) HANSEN, H. C. Influence of Temperature on the Curing of Cheddar Cheese. Can. Dairy Ice Cream J., **25** (6): 48, 50. 1946.

(7) HANSON, F. E., ARBUCKLE, W. S., AND SHEPARDSON, C. N. Factors Affecting the Quality of Southern Short Cure Cheddar Cheese. Texas Agr. Expt. Sta. Bull. 646. pp. 1–30. 1944.

(8) HUCKER, G. J., AND MARQUARDT, J. C. The Effect of Certain Lactic Acid Producing Streptococci upon the Flavor of Cheddar Cheese. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 117. 1926.

(9) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. J. Dairy Sci., **29**: 861–871. 1946.

(10) LANE, C. B. The Effect of Certain Bacteria on the Ripening of Cheddar Cheese Made from Pasteurized Milk. Iowa State College J. Sci., **9**: 173–175. 1934.

(11) LOCHRY, H. R. Curing Cheddar Cheese at Different Temperatures. Am. Butter Review, **9** (5): 18. 1947.

(12) MOJONNIER, T., AND TROY, H. C. Technical Control of Dairy Products. 1st ed. Mojonnier Bros. Co., Chicago, Ill. 1922.

(13) SANDERS, G. P., TITTSLER, R. P., AND WALTER, H. C. The Rapid Ripening of Cheddar Cheese Made from Pasteurized Milk. J. Milk Technol., **9** (3): 171–172. 1946.

(14) SWOPE, W. D. Testing Milk and Milk Products. School of Agr., Pennsylvania State College. Circ. 129. June, 1929. Rev. March, 1941.

(15) WILSON, H. L. Making American Cheddar Cheese of Uniformly Good Quality. USDA Mimeo. BDIM-947. 1942.

(16) WILSON, H. L., HALL, S. A., AND ROGERS, L. A. The Manufacture of Cheddar Cheese from Pasteurized Milk. J. Dairy Sci., **28**: 187–200. 1945.

(17) WOJTKIEWICZ, A., AND INIKKOFF, G. The Acceleration of the Ripening of Cheese. Tenth World's Dairy Congress, Rome-Milan. Section II. pp. 335–339. 1934.

THE SOLAR-ACTIVATED FLAVOR OF HOMOGENIZED MILK. V. THE ELECTROPHORETIC ANALYSIS OF A CONTRIBUTING MINOR-PROTEIN FRACTION[1]

BERNARD R. WEINSTEIN,[2] H. A. LILLEVIK, C. W. DUNCAN AND G. M. TROUT

Departments of Dairy, Chemistry and Agricultural Chemistry
Michigan Agricultural Experiment Station, East Lansing

It has been reported previously (6) that a minor-protein fraction isolated from skimmilk, after the major proteins had been removed, is capable of being photosensitized to produce the typical solar-activated flavor of homogenized milk. Furthermore, the elementary analysis and the percentage composition of the amino acids in the minor-protein fraction differed markedly from the other characterized whey proteins. Since the characterization and properties of this compound indicated that it was not a previously recognized whey protein, it was thought desirable to make an electrophoretic examination of the minor-protein fraction.

Various other investigators (1, 3, 4, 5) have studied whey proteins electrophoretically, but their methods of preparing the whey proteins varied considerably and none was comparable to our method of preparation.

The object of the present paper is to report the results obtained from an exploratory examination of the electrophoretic pattern of the minor-protein fraction and of the other whey protein fractions isolated during its preparation.

EXPERIMENTAL

Freshly separated skimmilk obtained from the Michigan State College Creamery was rennet-coagulated according to a procedure outlined previously (6). Figure 1 shows graphically the fractionation procedure employed in the isolation of the various whey protein fractions which were examined electrophoretically.

Electrophoretic analysis of the minor-protein fraction. One per cent solutions of the minor-protein fraction (fig. 1, fraction III) were prepared by dissolving a definite amount of four different buffer solutions and then dialyzing them to osmotic equilibrium at 5° C. for 24 hr., or until constant conductivity was reached on both sides of the membrane. The electrophoretic resolution of the minor-protein fraction in the various buffers was then studied at pH ranges from 3.3 to 9.3 with a Perkins-Elmer Tiselius electrophoresis apparatus at 1.2° C. (2). The electrophoretic patterns of unit magnification of both the ascending and descending boundaries obtained from the electrophoretic examination of the minor-protein fraction are illustrated in figure 2. The horizontal arrows

Received for publication March 5, 1951.

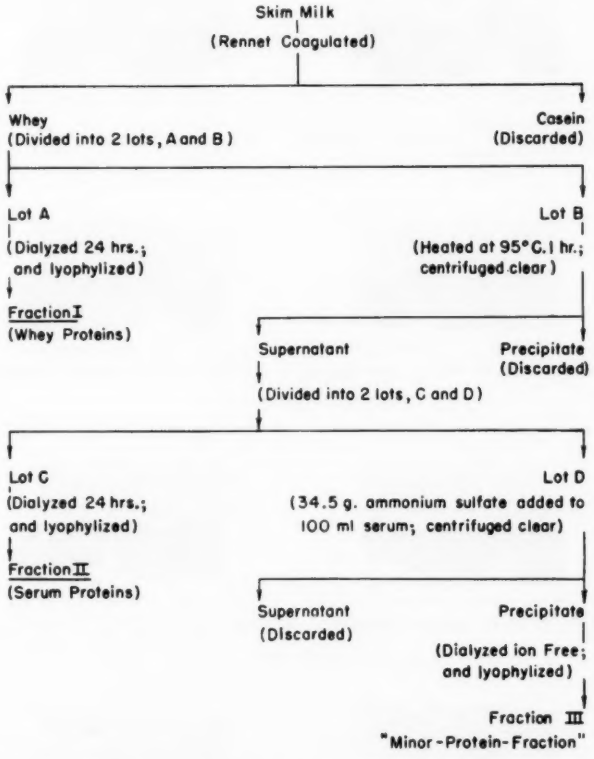
[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article no. 1227.

[2] This article is part of a dissertation presented to the Faculty of the Graduate School of Michigan State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

in figures 2 and 3 indicate the direction of migration. The diagrams on the left side of figures 2 and 3 represent ascending boundaries while those on the right represent descending boundaries. The tail of the arrow in both cases signifies the position of the starting boundary.

Electrophoretic analysis of whey proteins and heat-coagulated-whey serum proteins. In an effort to follow the various steps in the isolation of the minor-protein fraction electrophoretically, a series of patterns was obtained from the

Fig.1 FLOW SHEET DIAGRAM FOR FRACTIONATION OF MILK PROTEINS

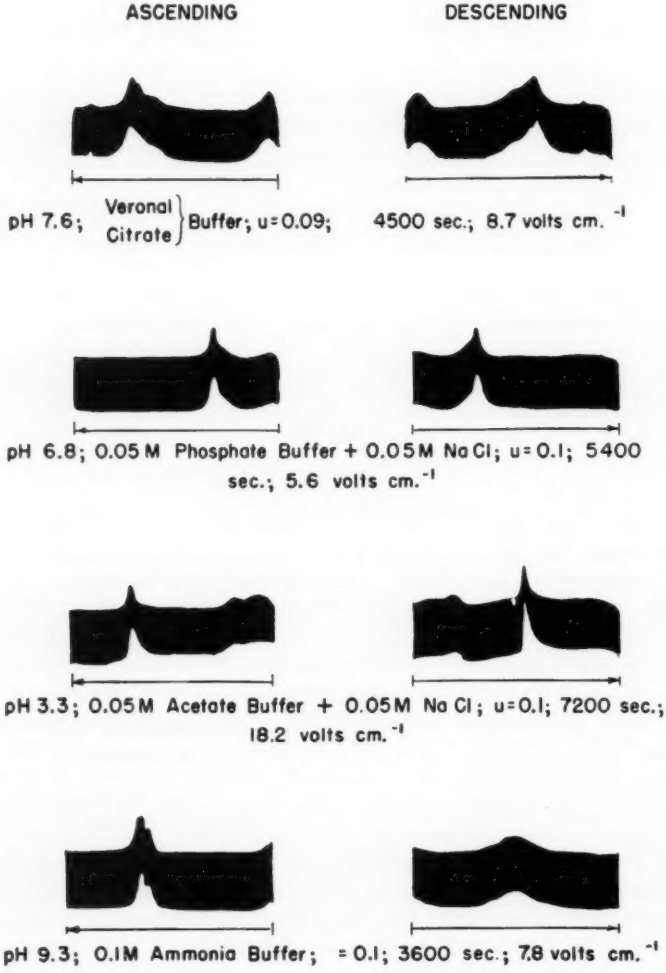


whey proteins and heat-coagulated-whey serum proteins. These two protein fractions were obtained in the course of the isolation of the minor-protein fraction and were removed and dried from the frozen state under high vacuum (fig. 1, fractions I and II). It should be mentioned that prior to lyophilization a precipitate formed during the dialysis of the whey against water and was discarded.

Samples of the whey proteins (fig. 1, fraction I) were made up in concentrations of 0.22, 0.24 and 1.0 per cent in three different buffer solutions and dialyzed to osmotic equilibrium at 5° C. for at least 24 hr.

The serum protein fraction (fig. 1, fraction II) was made up in 1 per cent concentration in veronal-citrate buffer and dialyzed as indicated above. The veronal-citrate buffer suggested by the work of Stanley *et al.* (5) was used be-

Fig.2 ELECTROPHORETIC PATTERNS FROM 1% SOLUTIONS OF MINOR-PROTEIN-FRACTION AT VARIOUS pH AND BUFFER MEDIA

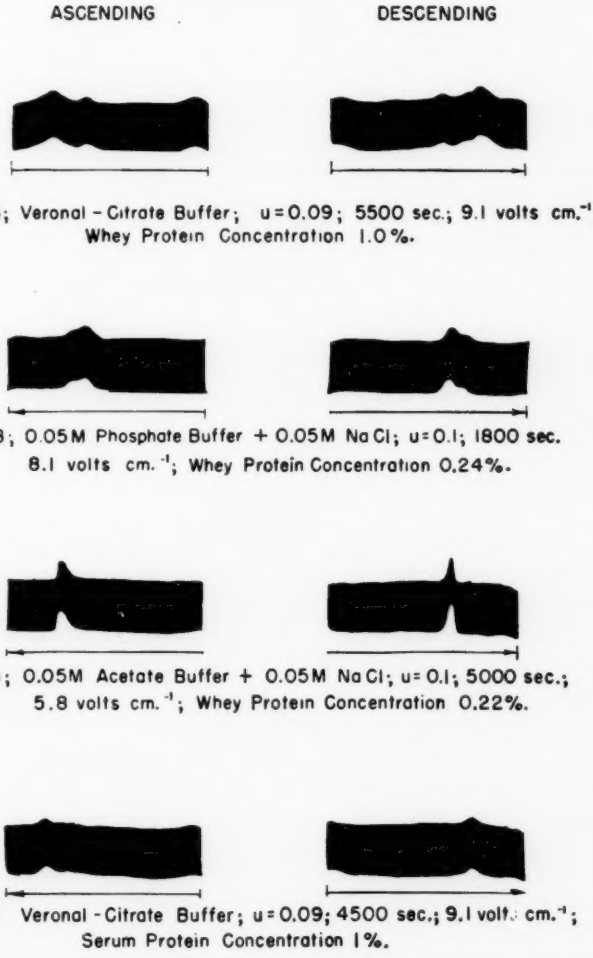

ASCENDING
DESCENDING
pH 7.6; Veronal Citrate Buffer; u=0.09; 4500 sec.; 8.7 volts cm.⁻¹
pH 6.8; 0.05 M Phosphate Buffer + 0.05 M Na Cl; u=0.1; 5400 sec.; 5.6 volts cm.⁻¹
pH 3.3; 0.05 M Acetate Buffer + 0.05 M Na Cl; u=0.1; 7200 sec.; 18.2 volts cm.⁻¹
pH 9.3; 0.1 M Ammonia Buffer; = 0.1; 3600 sec.; 7.8 volts cm.⁻¹

cause poor electrophoretic resolution was evident from the use of acetate and phosphate buffers (fig. 3).

The resulting patterns at unit magnification of both the ascending and de-

scending boundaries obtained from the electrophoretic analysis of the whey and heat-coagulated-whey serum proteins are shown in figure 3.

Fig. 3 ELECTROPHORETIC PATTERNS OF WHEY AND HEAT COAGULATED WHEY SERUM PROTEINS.


ASCENDING
DESCENDING
pH 7.6; Veronal - Citrate Buffer; u = 0.09; 5500 sec.; 9.1 volts cm.$^{-1}$; Whey Protein Concentration 1.0%.
pH 6.8; 0.05M Phosphate Buffer + 0.05M NaCl; u = 0.1; 1800 sec. 8.1 volts cm.$^{-1}$; Whey Protein Concentration 0.24%.
pH 3.3; 0.05M Acetate Buffer + 0.05M NaCl; u = 0.1; 5000 sec.; 5.8 volts cm.$^{-1}$; Whey Protein Concentration 0.22%.
pH 7.6 Veronal - Citrate Buffer; u = 0.09; 4500 sec.; 9.1 volts cm.$^{-1}$; Serum Protein Concentration 1%.

Any screening effect due to opalescence has been eliminated in the photographic reproductions of both figures.

DISCUSSION

It is evident from an inspection of the four patterns obtained for the minor-protein fraction that this fraction does not necessarily represent a homogeneous

compound (fig. 2). The curves seem to indicate that at least two components or complexes are present in the so-called minor-protein fraction. There was no pH range or buffer system used in this investigation that appeared to suggest otherwise. It is apparent, however, that the best resolution and enantiography for this fraction occurred when the veronal-citrate buffer system was used at pH 7.6 rather than at any other pH or buffer system. This conclusion also is evident from studies reported by Stanley *et al.* (5) and Deutsch (1).

Preliminary calculations showed that when mobility values of the major components were plotted as ordinates against the pH values of the buffer system as abscissas, an isoelectric zone of the minor-protein fraction was evident at pH 3.7 to 4.4. This range is in good agreement with the isoelectric range of 4.1 to 4.3 previously obtained by minimum solubility-pH relationships (6).

Inasmuch as this report is only intended to establish preliminary data on the electrophoretic character of the minor-protein fraction, it was not considered desirable at the present time to publish such measurements as individual mobilities and percentage composition for each component until further controlled work has been completed.

It should be pointed out that the patterns obtained on both the whey and heat-coagulated-whey serum proteins (fig. 3) in veronal-citrate buffer show conclusively that some of the components are lost after heat treatment of the whey. This would indicate that the heat-labile components of whey are relatively well separated by this fractionation procedure. It was mentioned previously that some precipitation occurred when the whey (fig. 1, fraction I) was dialyzed against water. The discarding of this precipitate may account for the smaller number of components in this whey fraction than would otherwise be anticipated.

The degree of resolution and enantiography was not affected noticeably by the concentration of the whey proteins when the veronal-citrate buffer was used. A preliminary pattern (not shown) of this fraction at a concentration of 0.24 per cent showed essentially the same resolution as that obtained when the concentration was 1 per cent.

A comparison of the electrophoretic pattern obtained at pH 7.6 in the veronal-citrate buffer of the serum protein (fraction II, fig. 3) with the pattern of the minor-protein fraction in the same buffer and at the same pH (fig. 2) shows that the two complexes are dissimilar.

SUMMARY

Electrophoretic analyses of the minor-protein fraction in various buffers and at various hydrogen-ion concentrations indicate that this fraction is composed of at least two components or complexes.

Best electrophoretic resolution and enantiographic patterns of all of the whey proteins were effected with veronal-citrate buffer at a pH of 7.6.

Electrophoretic patterns obtained with whey and heat-coagulated-whey serum proteins indicate that the heat labile whey components were effectively removed by the fractionation procedure employed in this work.

REFERENCES

(1) DEUTSCH, H. F. A Study of Whey Proteins from the Milk of Various Animals. J. Biol. Chem., **169**: 437–448. 1947.

(2) MOORE, D. H., AND WHITE, J. U. A New Compact Tiselius Electrophoresis Apparatus. Rev. Sci. Instruments, **19**: 700–706. 1948.

(3) SMITH, E. L. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. J. Biol. Chem., **165**: 665–676. 1946.

(4) SMITH, E. L. The Isolation and Properties of the Immune Proteins of Bovine Milk and Colostrum and their Role in Immunity: A Review. J. Dairy Sci., **31**: 127–138. 1948.

(5) STANLEY, W. G., ANDREWS, A. C., AND WHITNAH, C. H. Electrophoresis of Milk Proteins. I. Some Comparisons of Salt-Acid and Salt-Lyophilized Whey Fractions. J. Dairy Sci., **33**: 275–280. 1950.

(6) WEINSTEIN, B. R., DUNCAN, C. W., AND TROUT, G. M. The Solar-activated Flavor of Homogenized Milk. IV. Isolation and Characterization of a Whey Constituent Capable of Producing the Solar-activated Flavor. J. Dairy Sci., **34**: 570–576. 1951.

ELECTROPHORETIC STUDIES OF COMMERCIAL AND IODINATED CASEIN[1]

SYED KAMAL AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia

Casein, the chief protein in milk, was regarded in the earlier literature as the best example of a pure and homogeneous protein (6). Subsequently, solubility studies indicated that casein might be a mixture of proteins (10). It also was suggested, on the basis of solubility studies, that casein had two components. However, ultracentrifuge sedimentation studies showed that the various components of casein sedimented as a single constituent at an intermediate rate (15). This was thought to be due to very rapid establishment of equilibrium between single and associated molecules.

The first electrophoretic mobility studies of casein were made as early as 1929 (3). A few investigators subsequently have shown on the basis of electrophoretic studies, that casein consists of two main components α- and β-casein, and a third small γ component (12, 13, 15, 22). Hipp *et al.* (7) indicated that whole casein contains approximately 75 per cent α-, 22 per cent β- and 3 per cent γ-casein.

Many investigators have reported on the amino acid content of casein and particularly have shown the difference in the composition of α- and β-casein (1, 4, 5, 14).

The iodination of casein and its subsequent incubation results in the formation of a thyroactive protein. It is believed that an oxidative process, involving the coupling of two diiodotyrosine radicals and elimination of one side chain, occurs (8, 19). During the past few years, experiments on thyroprotein have been confined chiefly to studies on its biological activity and to its value in stimulating a mild hyperthyroid condition in farm animals.

Spectrographic absorption studies on iodinated protein indicated that these measurements were complicated by the fact that the influence of the various amino acids could not be differentiated because of their overlapping spectra (2) (see also discussion by Pitt-Rivers, 16). Other investigators reported that iodinated casein had its absorption maximum shifted toward longer wave lengths as compared with normal casein (17).

It also was reported that the X-ray diffraction pattern of iodinated casein was the same as that of iodine-free casein (20).

In view of the fact that casein is a heterogeneous protein, it was decided to study the electrophoretic pattern and mobilities of commercial and iodinated casein at progressive stages of iodination and incubation.

Received for publication March 8, 1951.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1171.

EXPERIMENTAL

Commercial casein was iodinated according to the procedure described by Reineke and Turner (18). A commercial iodinated casein known as Protamone also was used in these experiments.[2]

Commercial casein and Protamone were dissolved in suitable amounts of appropriate buffers and dialyzed against the same buffers in cellophane bags at 4° C. for electrophoretic studies. Dialysis was considered complete when there was no change in the conductance of the buffer solution after remaining in contact with the dialyzing bag for 12 hr. Aliquot samples of iodinated casein were taken for dialysis for electrophoretic studies after stirring, after iodination at 39° C. and after stirring and incubation at 70° C. subsequent to iodination.

Conductivity measurements were made by the use of a model K_2 potentiometer in conjunction with an audio-oscillator, resistance box, conductivity cell and earphones.

Electrophoretic studies were made in a new Tiselius electrophoresis apparatus model 38 manufactured by Perkin-Elmer Corporation, Glenbrook, Conn. It incorporates a 2-ml.-capacity Tiselius cell with an optical system based on Longsworth's scanning modification of the Toepler-Schlieren method.

The measurements of potential gradient and electrophoretic mobility were made according to the procedures described by Longsworth and MacInnes (11). The electrophoretic mobilities were measured on the descending patterns only and open type buffer tubes have been used in conjunction with a syringe for compensation.

RESULTS

In an electrophoretic study of commercial casein in phosphate, barbiturate and acetate buffers at various hydrogen ion concentrations, it was observed that casein shows two principle peaks (figs. 1A to 4A). The large peak represents alpha-casein and the small one represents beta-casein. The descending pattern of casein in barbiturate buffer at a pH of 8.3 with an ionic strength of 0.1 also shows a very small third peak (fig. 2A). The ascending pattern of casein in barbiturate buffer at pH's of 8.3 and 7.5 with an ionic strength of 0.1 also shows a small sharp peak in the alpha casein component (figs. 2A and 3A). Casein stirred vigorously at pH 8.0 at 39° C. for 4 hr. shows two well-defined peaks in barbiturate buffer pH 7.5 with an ionic strength of 0.1 (fig. 5A). However, subsequent to incubation and stirring of casein at 70° C. for 18 hr. only one peak was observed in barbiturate buffer at a pH of 7.5 with an ionic strength of 0.1 (fig. 5B).

In an electrophoretic study of thyroprotein in phosphate, barbiturate and acetate buffers at various hydrogen ion concentration, it was observed that thyroprotein shows a single peak (figs. 1B and 4B). The patterns of thyroprotein in phosphate and acetate buffers are not well defined and symmetrical, indicating the unsuitability of these buffers for the electrophoretic study of thyroprotein (figs. 1B and 4B). Casein after iodination with stirring at pH 8.0 and

[2] Kindly supplied by W. R. Graham, Jr., Cerophyl Lab., Kansas City, Mo.

39° C. shows a very small peak of β-casein in the descending pattern in barbiturate buffer at a pH of 7.5 with an ionic strength of 0.1. A faint diffuse peak of β-casein is observed in the corresponding ascending pattern (fig. 6A). However, after incubation with stirring at 70° C. subsequent to iodination of casein,

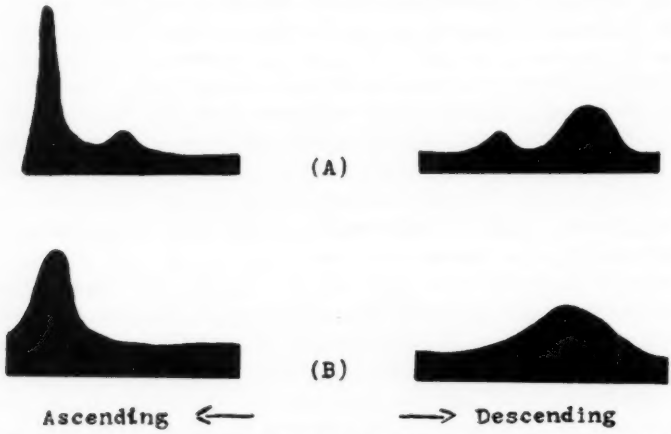


FIG. 1. Electrophoretic patterns obtained in phosphate buffer (0.02 *M* Na_3PO_4 and 0.15 *M* NaCl) at a pH of 7.4. (A) Casein, 0.902% protein after electrophoresis for 3,000 sec. at field strength of 10.505 volts/cm. (B) Protamone, 0.904% protein after electrophoresis for 2,400 sec. at a field strength of 10.505 volts/cm.

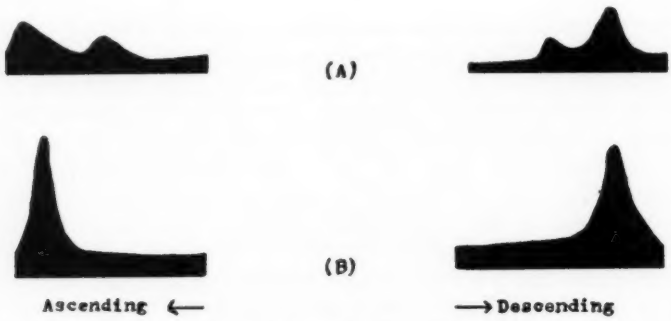


FIG. 2. Electrophoretic patterns obtained in barbiturate buffer (0.1 *M* Li diethyl barbiturate) at a pH of 8.3 with an ionic strength of 0.1. (A) Casein, 1.239% protein after electrophoresis for 4,620 sec. at a field strength of 11.393 volts/cm. (B) Protamone, 1.014% protein after electrophoresis for 4,380 sec. at a field strength of 11.393 volts/cm.

only a single peak is observed in barbiturate buffer at a pH of 7.5 with an ionic strength 0.1 (fig. 6B).

The electrophoretic mobility of α-casein in acetate and barbiturate buffers at pH of 6.0, 7.5 and 8.3 with an ionic strength of 0.1 was -4.8^{10-5}, -8.897^{10-5} and -4.80^{10-5}, respectively. The electrophoretic mobility of beta casein was -1.726^{10-5},

-4.337^{10-5} and -2.944^{10-5}, whereas that of thyroprotein was -6.0^{10-5}, -9.231^{10-5} and -6.653^{10-5} under the same conditions (table 1). The electrophoretic mobility of casein after incubation at 70° C. in barbiturate buffer pH 7.5 with an ionic strength of 0.1 was observed to be -7.369^{10-5}, whereas after iodination and incubation it increased to -8.943^{10-5} under the same conditions.

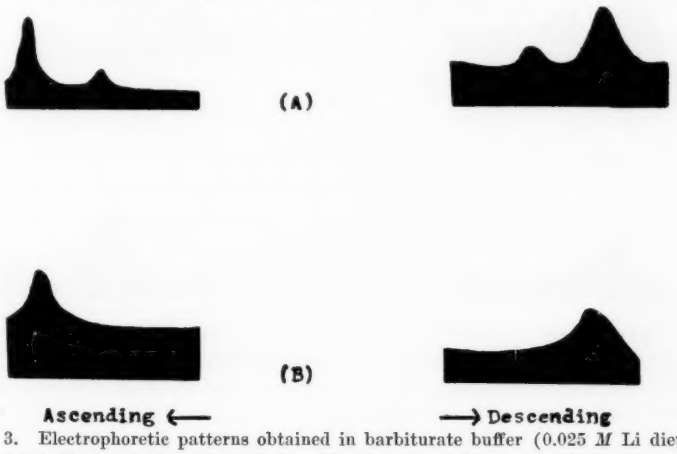


FIG. 3. Electrophoretic patterns obtained in barbiturate buffer (0.025 *M* Li diethyl barbiturate, 0.025 *M* LiCl, 0.025 *M* barbituric acid) at a pH of 7.5 with an ionic strength of 0.1. (A) Casein, 1.25% protein after electrophoresis for 2,700 sec. at a field strength of 12.156 volts/cm. (B) Protamone, 1.15% protein after electrophoresis for 2,940 sec. at a field strength of 12.156 volts/cm.

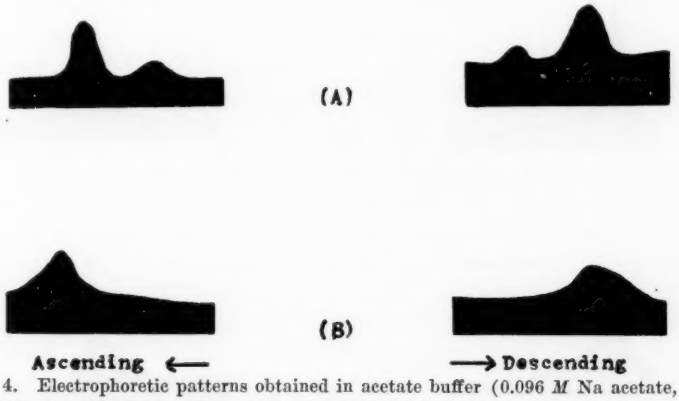


FIG. 4. Electrophoretic patterns obtained in acetate buffer (0.096 *M* Na acetate, 0.004 *M* acetic acid) at a pH of 6.0 with an ionic strength of 0.1. (A) Casein, 0.318% protein after electrophoresis for 4,500 sec. at a field strength of 10.554 volts/cm. (B) Protamone, 0.375% protein after electrophoresis for 4,500 sec. at a field strength of 10.554 volts/cm.

DISCUSSION

The electrophoretic pattern of commercial casein showing two principal components α- and β-casein observed in these studies (figs. 1A and 4A) are in ac-

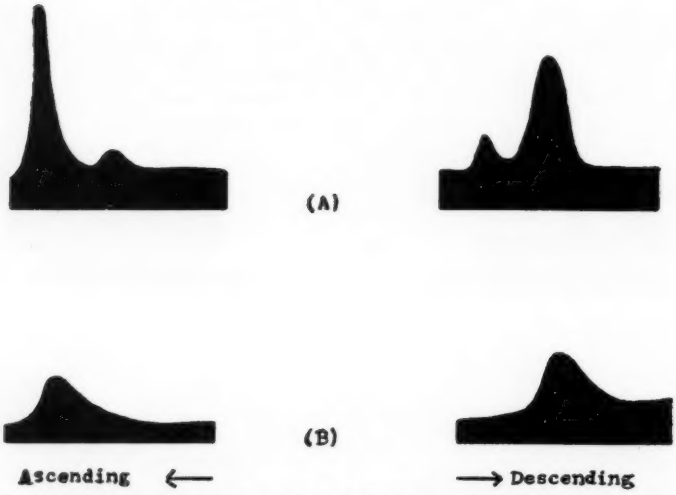


FIG. 5. Electrophoretic patterns obtained in barbiturate buffer (0.025 *M* Li diethyl barbiturate, 0.025 *M* LiCl 0.025 *M* barbituric acid) at a pH of 7.5 with an ionic strength of 0.1. (A) Casein, 1.238% protein after stirring at 39° C. for 4 hr. without iodine, after electrophoresis for 2,700 sec. at a field strength of 12.156 volts/cm. (B) Casein, 0.966% protein after incubation at 70° C. for 18 hr. without iodine after electrophoresis for 2,400 sec. at a field strength of 12.156 volts/cm.

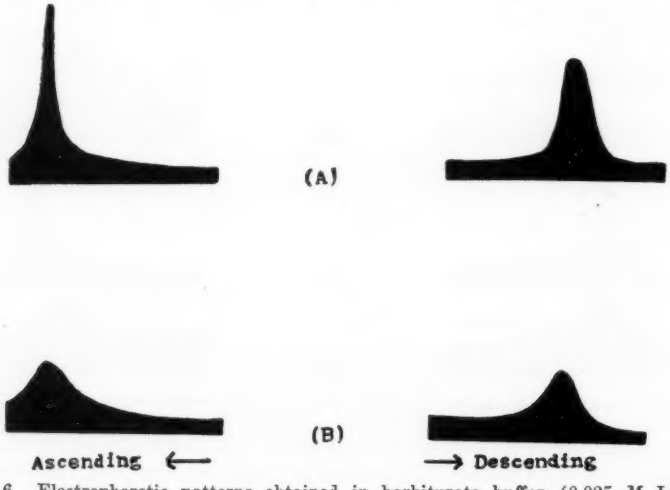


FIG. 6. Electrophoretic patterns obtained in barbiturate buffer (0.025 *M* Li diethyl barbiturate, 0.025 *M* LiCl, 0.025 *M* barbituric acid) at a pH of 7.5 with an ionic strength of 0.1. (A) Casein, 1.053% protein, after iodination at 39° C. for 4 hr. after electrophoresis for 2,700 sec. at a field strength of 12.156 volts/cm. (B) Casein, 0.862% protein incubated at 70° C. for 18 hr. after iodination, after electrophoresis for 2,400 sec. at a field strength of 12.156 volts/cm.

TABLE 1

Mobilities of casein components and thyroprotein at an ionic strength of 0.1 mobility ($cm.^2$, $volt^{-1}$, $sec.^{-1} \times 10^{-4}$) at pH:

	7.5	8.3	6.0
Buffer	Barbiturate	Barbiturate	Acetate
α-Casein	−8.897	−5.604	−4.80
β-Casein	−4.337	−2.944	−1.726
Protamone	−9.231	−6.653	−6.00
Casein after incubation at 70° C.	−7.369	.....	.....
Casein after iodination and incubation	−8.943	.....	.....

cord with the work of other investigators (12, 13, 15, 22). The small third peak observed in the descending pattern of casein in barbiturate buffer at pH 8.3 with an ionic strength of 0.1 (fig. 2A) is different from the gamma peak observed by other investigators (9, 12) because it has no counterpart in the ascending pattern. It also is different from a small faster diffuse boundary observed by Warner (22) in the descending electrophoretic pattern of casein because it is the slowest moving boundary. The ascending pattern of casein in barbiturate buffer at a pH of 8.3 and 7.5 with an ionic strength of 0.1 also shows a small sharp peak (figs. 2A and 3A), as observed by Warner (22) in the ascending electrophoretic pattern of casein in barbiturate buffer at a pH 7.78 with a protein concentration of 0.6 per cent.

In these studies the additional small peak in the alpha fraction was observed in the ascending electrophoretic pattern of casein in barbiturate buffer at a pH of 8.3 and 7.5 at a protein concentration of more than 1 per cent. Warner (22), however, reported that the mobilities of the two peaks in the alpha fraction became low as the protein concentration was increased until only one peak was evident at a concentration of 1 per cent.

The electrophoretic pattern of casein after heating with stirring at 39° C. for 4 hr. in barbiturate buffer at pH 7.5 with ionic strength of 0.1 showing two distinct peaks (fig. 5A) indicates that this treatment did not affect the electrophoretic pattern of casein. However, when the sample subsequently is heated at 70° C. for 18 hr., it shows a single peak (fig. 5B). This observation is in accord with those of other investigators (21) who reported that heating casein solution above 40° C. converted the casein into molecules of higher molecular weight (188,000).

The electrophoretic patterns of thyroprotein in phosphate, barbiturate and acetate buffers with various hydrogen ion concentrations indicate that thyroprotein is homogeneous electrophoretically and thus is different from casein from which it is prepared by iodination under specific conditions (figs. 1B to 4B). This is believed to be due to the formation of a single component through polymerization of the two components. However, iodination with stirring of casein at 39° C. does not complete this process and a small peak of β-casein is still observed in the electrophoretic pattern in barbiturate buffer pH 7.5 with an ionic strength of 0.1 (fig. 6A). However, subsequent incubation of iodinated

casein with stirring at 70° C. completes the process of polymerization and a single peak is observed in the electrophoretic pattern in barbiturate buffer at pH 7.5 with an ionic strength of 0.1 (fig. 6B).

The electrophoretic mobility of the α-casein fraction observed was -4.8 to -8.897^{10-5} and that of β-casein -1.726 to -4.337^{10-5} in acetate and barbiturate buffers at pH 6.0, 7.5 and 8.3 with an ionic strength of 0.1 (table 1). Warner (22) reported the electrophoretic mobility of α-casein to be -6.16 to -7.52^{10-5} and that of β-casein as -1.6 to -3.27^{10-5}. These differences in observations of electrophoretic mobility are due to the different hydrogen ion concentrations of the buffers used and the temperature at which the studies were made. Furthermore, these observations of electrophoretic mobility were made on separated α- and β-casein fractions.

The electrophoretic mobility of thyroprotein observed was -6.00^{10-5}, -6.653^{10-5} and -9.231^{10-5} in acetate and barbiturate buffers at pH 6.0, 8.3 and 7.5, respectively, with an ionic strength of 0.1 (table 1). Thus, it is observed that the electrophoretic mobility of thyroprotein is significantly higher than that of either the alpha- or beta-casein fraction. It was observed that the electrophoretic mobility of casein after incubation at 70° C. was -7.4^{10-5} and after iodination and incubation -8.9^{10-5}, indicating that iodination causes a significant change in the electrophoretic mobility of casein, possibly due to the rearrangements of groups in the casein molecules. The difference in observation of electrophoretic mobility of iodinated and the commercial product, Protamone, may be attributed to the difference in method of preparation.

SUMMARY

An electrophoretic study of commercial casein, iodinated casein, and Protamone was made in a new model no. 38 electrophoresis apparatus (Perkin-Elmer Corp.).

It was observed that casein consists of an α and β component. The observations of other investigators of the electrophoretic pattern of casein were confirmed.

It was observed that Protamone, unlike its mother substance casein, was homogeneous electrophoretically.

The electrophoretic mobility of Protamone was -6.0 to -9.2^{10-5} at pH 6.0, 8.3 and 7.5 in acetate and barbiturate buffers with an ionic strength of 0.1. Furthermore, the electrophoretic mobility of Protamone was significantly higher than either α- or β-casein under all the conditions studied.

It also was observed that incubation with stirring of casein at 39° C. did not change the electrophoretic pattern in barbiturate buffer pH 7.5 with an ionic strength of 0.1. However, when iodine was added the electrophoretic pattern was changed and only a small peak of β-casein was observed.

Casein incubated at 70° C. with or without iodination when studied electrophoretically in barbiturate buffer pH 7.5 with an ionic strength of 0.1 showed a single peak.

REFERENCES

(1) Block, R. J., and Bolling, D. *The Amino Acid Composition of Proteins and Foods.* Springfield, Ill. Charles C. Thomas. 1945.

(2) Feraud, K., Dunn, M. S., and Kaplan, J. Spectroscopic Investigations of Amino Acids and Amino Acid Derivatives. J. Biol. Chem., **112**: 323. 1935.

(3) Fridoux, E. B. R., and Howitt, F. O. The Electrophoresis of Protein Solution in the Presence of Gold Solution, Albumin, Gelatin, and Casein. Proc. Roy. Soc. A., **126**: 126. 1929.

(4) Gordon, W. G., Semmett, W. F., Cable, R. S., and Morris, M. Amino Acid Composition of Alpha Casein and Beta Casein. J. Am. Chem. Soc., **71**: 3293. 1949.

(5) Groh, J., Kardos, E., Denes, K., and Serenyi, V. Fractionation of Casein. Z. physiol. Chem., **226**: 32. 1934.

(6) Hammarsten, O. Z. Ueber den gehalt das Casein an Schwefel und uber die Bestimmung des Schwefels in protein substansen. Z. physiol. Chem., **9**: 273. 1885.

(7) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of Gamma Casein. J. Am. Chem. Soc., **72**: 4928. 1950.

(8) Johnson, T. B., and Tewkesbury, L. B. The Oxidation of 3,5-diiodothyrosine to Thyroxine. Proc. Natl. Acad. Sci. Wash., **28**: 73. 1942.

(9) Krejci, L. E., Jennings, R. K., and Smith, L. D. Electrophoretic Evidence for Complex Formation in Casein. J. Franklin Inst., **232**: 592. 1941.

(10) Linderstrom-Lang, K. Individuality of Casein. Preliminary paper. Z. physiol. Chem., **176**: 76. 1928.

(11) Longsworth, L. G., and MacInnes, D. A. The Interpretation of Simple Electrophoretic Pattern. Am. Chem. Soc. J., **62**: 705. 1940.

(12) Mellander, O. Electrophoretic Studies on Casein. Biochem. Z., **300**: 240. 1939.

(13) Mellander, O. Caseins from Human and Cow's Milk and their Behavior on Hydrolysis with Different Proteolytic Enzymes. Acta. Paediat., **32**: 668. 1945.

(14) Osborne, T. B., and Wakeman, A. J. Some New Constituents of Milk. III. A New Protein Soluble in Alcohol. J. Biol. Chem., **33**: 243. 1918.

(15) Philpot, F. F., and Philpot, J. St. L. The Effect of Calcium on the Sedimentation Constant of Casein. Proc. Roy. Soc. B., **127**: 21. 1939.

(16) Pitt-Rivers, R. *The Chemical Assay of Thyroxine and other Substances with Thyroid Activity.* Chap. XX. *Hormone Assay.* Ed. by C. W. Emmens, Pub. Academic Press, N. Y. 1950.

(17) Reineke, E. P., and Turner, C. W. Formation *in vitro* of Highly Active Thyroproteins, their Biologic Assay and Practical Use. Mo. Agr. Expt. Sta. Bull. 355. 1942.

(18) Reineke, E. P., and Turner, C. W. The Effect of Manganese Compounds and Certain other Factors on the Formation of Thyroxine in Iodinated Casein. J. Biol. Chem., **161**: 613. 1945.

(19) Reineke, E. P., Williamson, M. B., and Turner, C. W. The Effect of Progressive Iodination Followed by Incubation at High Temperature on the Thyroidal Activity of Iodinated Proteins. J. Biol. Chem., **147**: 115. 1943.

(20) Spiegel, M., Hamilton, R. H., Jr., and Henry, G. C. X-ray Diffraction Studies in Iodinated Amino Acids and Proteins. Biochem. J., **36**: 825. 1942.

(21) Svedberg, T., Carpenter, L. M., and Carpenter, D. C. The Molecular Weight of Casein. Am. Chem. Soc. J., **52**: 701. 1930.

(22) Warner, R. C. Separation of Alpha and Beta Casein. J. Am. Chem. Soc., **66**: 1725. 1944.

ELECTROPHORETIC STUDY OF BOVINE THYROGLOBULIN AND IODINATED BOVINE GAMMA GLOBULIN[1]

SYED KAMAL AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia

Studies in the past by various investigators on thyroglobulin have been confined chiefly to its iodine (2, 4, 6, 9, 13), thyroxine (7, 13, 16, 19) and amino acid and nitrogen contents (1, 4, 9, 18). A few studies on its isoelectric point (9, 14), molecular weight (9), ultraviolet absorption curves (5) and fluorescence (3) have been reported also.

The molecular weight of hog thyroglobulin has been reported to vary between 650,000 and 700,000, as calculated from the sedimentation and diffusion constants (4, 9). The molecular weight of bovine gamma globulin, the heaviest blood protein, was reported to be only 175,000 (10). Since Derrien *et al.* (4), reported that the amino acids of thyroglobulin from pigs and cattle are always the same, the molecular weight of bovine thyroglobulin would be expected to be similar to that of hog thyroglobulin. The great difference in the molecular weight of bovine serum globulin and thyroglobulin suggests that in addition to the iodination of the tyrosine in the thyroid gland there also is condensation or polymerization of the blood proteins.

Apparently, electrophoretic study of thyroglobulins has not been reported. The question whether the preparations consist of one or more components has not been answered. Recently, it was shown by electrophoretic study that bovine gamma globulin consists of two components which can be separated by chemical methods (10). It seemed of interest to study bovine thyroglobulin electrophoretically in order to determine the number of components. It also was considered important to note the changes in the electrophoretic pattern and mobility of bovine gamma globulin before and after iodination and incubation according to the method of Reineke and Turner (15).

EXPERIMENTAL

Fresh bovine thyroid glands were obtained packed in dry ice from Swift & Co., Kansas City, and stored at $-10°$ C. Thyroglobulin was prepared mainly according to Heidelberger and Palmer (8), and, after final precipitation with $(NH_4)_2\ SO_4$, was dissolved in water and dialyzed against distilled water in a cellophane bag at 4° C. After dialysis, samples were taken for electrophoretic studies, the remaining solution being lyophilized. Individual samples were dialyzed against acetate or barbiturate buffers with an ionic strength of 0.1 at pH levels of 6.0, 7.5 and 8.3. Dialysis was considered complete when the conductance of the buffer was unchanged after 12 hr. The presence of blood pigments in the thyroglobulin solution after dialysis made it necessary to dilute the

Received for publication March 8, 1951.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1170.

samples for electrophoretic analysis slightly below 1 per cent protein concentration.

In the case of acetate buffer with a pH of 6.0, the poor solubility of thyroglobulin was an additional factor in reducing the protein concentration still more. Nitrogen determinations were made by the micro-Kjeldahl method. The amount of nitrogen per 100 ml. of sample was multiplied by 6.25 to obtain the protein concentration.

Fraction II (gamma globulin) from bovine plasma obtained from Armour Laboratories, Chicago, was studied electrophoretically in barbiturate buffer at a pH of 8.3 with an ionic strength of 0.1. This material was iodinated according to the procedure described by Reineke and Turner (15). Aliquot samples were obtained both after iodination and after incubation at 70° C. subsequent to iodination and electrophoretically studied in barbiturate buffer at a pH of 8.3 with an ionic strength of 0.1.

Electrophoretic studies were made according to the method of Tiselius (17), in a new electrophoresis apparatus model 38 manufactured by the Perkin-Elmer Corporation, Glenbrook, Connecticut. It incorporates a 2-ml. capacity Tiselius cell and has an optical system based on Longsworth's scanning modification of the Toepler-Schlieren method.

Buffer solutions used were prepared according to those described by Longsworth (11). Conductivity water used in these experiments was prepared from single-distilled water in a small quartz-distilling apparatus. The electrophoretic mobility measurements were made in the descending patterns only, according to the procedure described by Longsworth and MacInnes (12). Open-type buffer tubes in conjunction with a syringe as a compensator were used in these experiments.

RESULTS

In an electrophoretic study of bovine thyroglobulin in acetate and barbiturate buffers at various hydrogen ion concentrations with an ionic strength of 0.1, it was observed that bovine thyroglobulin has a single peak (figs. 1A, 1B and 2A). It indicates that bovine thyroglobulin is electrophoretically homogeneous under all the conditions studied.

The mobilities of bovine thyroglobulin observed were -3.6^{10-5}, -6.8^{10-5} and -5.3^{10-5} at pH of 6.0, 7.5 and 8.3, respectively, in acetate and barbiturate buffers at 1° C. with an ionic strength of 0.1.

The electrophoretic mobility of fraction II from bovine plasma in barbiturate buffer at a pH of 8.3 with an ionic strength of 0.1 was -3.7^{10-5} at 1° C., whereas after iodination it was -3.7^{10-5} and -4.3^{10-5} after incubation at 70° C. subsequent to iodination under the same condition (figs. 2B, 3A and 3B).

DISCUSSION

Bovine thyroglobulin prepared by the procedure described was electrophoretically homogeneous under all conditions of study. These observations are in agreement with those of Heidelberger and Palmer (8) on hog thyroglobulin.

These workers fractionated hog thyroglobulin into three fractions by precipitation with Na_2So_4 and found that these fractions were identical on the basis of

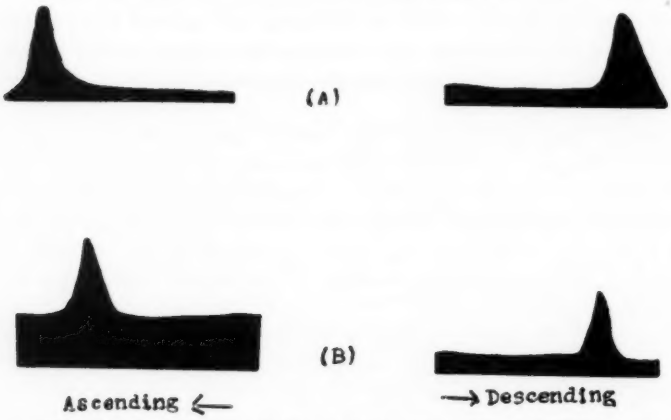


FIG. 1. Electrophoretic pattern obtained of bovine thyroglobulin 0.629% protein: (A) In barbiturate buffer (0.025 *M* LiCl) at a pH of 7.5 with an ionic strength of 0.1 after electrophoresis for 3,960 sec. at a field strength of 12.156 volts/cm. (B) In barbiturate buffer (0.1 *M* Li diethyl barbiturate) at a pH of 8.3 with an ionic strength of 0.1, after electrophoresis for 4,320 sec. at a field strength of 11.993 volts/cm.

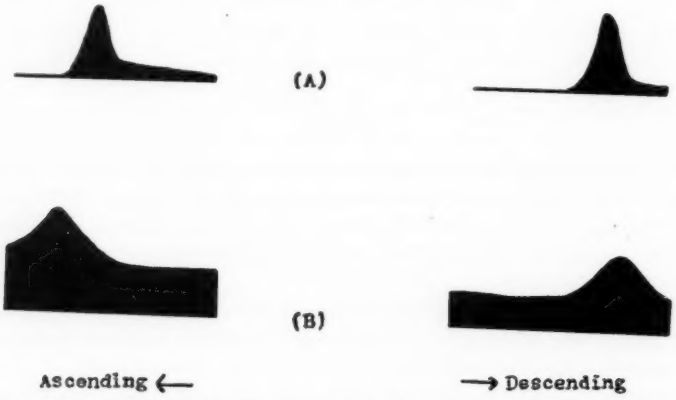


FIG. 2. (A) Electrophoretic pattern obtained of bovine thyroglobulin 0.476% protein in acetate buffer (0.096 *M* sodium acetate and 0.004 *M* acetic acid) at a pH of 6.0, with an ionic strength of 0.1 after electrophoresis for 6,840 sec. at a field strength of 10.554 volts/cm. (B) Electrophoretic pattern obtained of bovine plasma fraction II 1.231% protein in barbiturate buffer (0.1 *M* Li diethyl barbiturate) at a pH of 8.3 with an ionic strength of 0.1 after electrophoresis for 7,020 sec. at a field strength of 11.993 volts/cm.

chemical analysis. Furthermore, electrophoretic mobility and sedimentation constant studies of hog thyroglobulin by Heidelberger and Pedersen (9) also indicated the homogeneity of hog thyroglobulin.

The electrophoretic mobility measurements of hog thyroglobulin, reported by Heidelberger and Pedersen (9), varied from 4.1 to 19.6 at pH of 3.27 to 8.7 at a temperature of 20° C. These differences in observations are due mainly to the difference in temperature and the type of apparatus used when the observations were made.

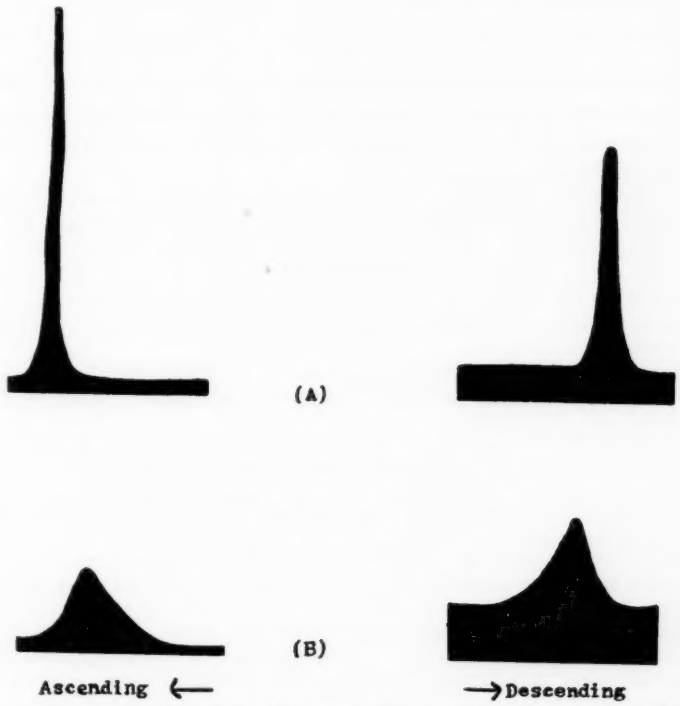


FIG. 3. Electrophoretic pattern obtained of bovine plasma fraction II in barbiturate buffer (0.1 *M* Li diethyl barbiturate). (A) After iodination with stirring at 39° C. for 4 hr., gamma globulin 1.446% protein, after electrophoresis for 6,720 sec. at a field strength of 11.993 volts/cm. (B) Bovine plasma fraction II 1.474% protein incubated at 70° C. with stirring subsequent to iodination, after electrophoresis for 5,040 sec. at a field strength of 11.993 volts/cm.

The electrophoretic mobility of fraction II from bovine plasma in barbiturate buffer at a pH of 8.3 with an ionic strength of 0.1 at 1° C. was observed to be -3.7^{10-5}. However, the electrophoretic mobility of bovine gamma$_1$ globulin, either in veronal buffer with an ionic concentration of 0.1 with pH 8.6 or veronal citrate buffer with ionic strength of 0.088 at a pH of 8.6, has been reported to be 2.1×10^{-5} cm.^{2}/sec./volt (10). The mobility of bovine gamma$_2$ globulin fraction has been reported to be 1.25×10^{-5} cm.^{2}/sec./volt under the same condition. Conceivably the electrophoretic mobility of fraction II from bovine plasma differs from both bovine gamma$_1$ and gamma$_2$ globulin fraction because of the

treatment to which it has been subjected during the process of preparation, if it represents the same fractions.

However, there is a definite increase in the electrophoretic mobility of fraction II from bovine plasma after iodination and incubation at 70° C. This change may be explained by rearrangements of groups within the protein molecule due to iodination and incubation.

SUMMARY

Bovine thyroglobulin prepared as described was found to be electrophoretically homogeneous at pH 6.0, 7.5 and 8.3 in acetate and barbiturate buffers with an ionic strength of 0.1. Its electrophoretic mobility varied from -3.6^{10-5} to -6.8^{10-5} under the same conditions.

The electrophoretic mobility of fraction II from bovine plasma after iodination and subsequent incubation at 70° C. increased from -3.7^{10-5} to -4.3^{10-5}.

REFERENCES

(1) Block, R. J., and Bolling, D. A Note on the Amino Acids Yield by Yeast, Sunflower Seed Meal and Sesame Seed after Hydroylsis of the Fat-free Tissue. Arch. Biochem., **6**: 277. 1945.

(2) Cavett, J. W., Rice, C. O., and McClendon, J. F. Thyroglobulin Studies. 1. The Thyroxine and Iodine Content of Normal and Goitrous Human Thyroglobulin. J. Biol. Chem., **110**: 673. 1935.

(3) Dempsey, E. W. Fluorescent and Histochemical Reactions in the Rat Thyroid Gland at Different States of Physiological Activity. Endocrinol., **34**: 27–38. 1944.

(4) Derrien, Y., Michel, R., Pedersen, K. O., and Roche, J. Recherches sur la préparation et sur les proprietés de la thyroglobuline pure. I. Biochimica et biophysica acta, **2**: 454–470. 1948. II. **3**: 436–441. 1949.

(5) Gersh, I., and Baker, R. F. Total Protein and Organic Iodine in the Colloid of Individual Follicles of the Thyroid Gland of the Rat. J. Cell. and Comp. Physiol., **21**: 213. 1943.

(6) Harrington, C. R. *The Thyroid Gland.* Oxford Univ. Press. London. 1933.

(7) Harrington, C. R., and Randall, S. S. Observations on the Iodine-containing Compounds of the Thyroid Gland. Isolation of DL-3:5-Diiodotyrosine. Biochem. J., **23**: 373. 1929.

(8) Heidelberger, M., and Palmer, W. W. The Preparation and Properties of Thyroglobulin. J. Biol. Chem., **101**: 433. 1933.

(9) Heidelberger, M., Pedersen, K. O. The Molecular Weight and Isoelectric Point of Thyroglobulin. J. Gen. Physiol., **19**: 95. 1935.

(10) Hess, L. G., and Deutsch, H. F. Biophysical Studies of Blood Plasma Proteins. VIII. Separation and Properties of the Gamma-globulin of the Serum of Normal Cows. J. Am. Chem. Soc., **70**: 84. 1948.

(11) Longsworth, L. G. Recent Advances in the Study of Proteins by Electrophoresis. Chemical Rev., **30**: 323. 1942.

(12) Longsworth, L. G., and MacInnes, D. A. The Interpretation of Simple Electrophoretic Pattern. J. Am. Chem. Soc., **62**: 705. 1940.

(13) Michel, R., and Lafon, M. Iodine and Thyroxine Content of Thyroglobulin. Compt. rend. soc. biol., **140**: 634. 1946.

(14) Rawson, R. W., Grahm, R. M., and Riddell, C. B. Physiological Reactions of Thyroid-stimulating Hormone of the Pituitary. II. The Effect of Normal and Pathological Human Thyroid Tissues on the Activity of the Thyroid-stimulating Hormone. Ann. Internal Med., **19**: 405. 1944.

(15) REINECKE, E. P., AND TURNER, C. W. The Effect of Manganese Compounds and Certain Other Factors on the Formation of Thyroxine in Iodinated Casein. J. Biol. Chem., **161**: 613. 1945.

(16) ROCHE, J., MICHEL, R., AND LAFON, M. Tyrosine, Tryptophan, Iodine, and Thyroxine Contents of Thyroglobulin from Various Animals. Compt. rend. soc. biol., **141**: 301–303. 1947.

(17) TISELIUS, A. A New Apparatus for Electrophoretic Analysis of Colloidal Mixtures. Trans. Faraday Soc., **33**: 524. 1937.

(18) WHITE, A., AND GORDON, W. G. Basic Amino Acids Yielded by Thyroglobulin. Proc. Soc. Exptl. Biol. Med., **32**: 354. 1935.

(19) WOOLF, J., AND CHAIKOFF, I. L. The Relation of Thyroxine to Total Iodine in the Thyroid Gland. Endocrinol., **41**: 295. 1947.

PERMANENT PIPE LINES FOR DAIRY PLANTS[1]

DONALD R. MOORE, P. H. TRACY AND Z. JOHN ORDAL
Department of Food Technology, University of Illinois, Urbana

Modern dairy plant equipment has been designed to permit complete dismantling for thorough cleaning and sanitizing. Such procedures, however, are time consuming and frequently result in damaged pipes and fittings. Because of this, considerable interest is being shown at the present time in the possibility of using permanent pipe lines in dairy plants.

The use of permanent pipe lines is not new to the food industry. For several years certain breweries have used sealed copper pipe lines through which wort is pumped daily (8). It has been reported (6) that a large soup manufacturer uses a stainless steel pipe to conduct vegetable juices from one department to another. Another vegetable processing company uses a pyrex glass pipe line for the same purpose (4). In none of these cases are the pipe lines taken apart for cleaning. Fisker (2) reports that Danish dairies have used pipe lines specially designed for flush washing since 1937. Certain dairy operators in Holland have followed similar procedures (7). At present, a number of milk companies in various sections of this country are experimenting with permanent pipe lines (1, 3, 9, 10).

To further study this problem, a permanent pipe line consisting of both pyrex glass and stainless steel was installed in the University of Illinois dairy plant. It was planned that merits of this system were to be judged on the basis of the sanitary condition of the line and the bacteriological condition of the milk passing through it.

EXPERIMENTAL PROCEDURE

Description of the permanent pipe line. The permanent pipe line was designed in accordance with agreements reached at a conference of dairy engineers, public health authorities and food technologists. One of the factors to be investigated was the importance of inside smoothness of the pipe. Therefore, pyrex glass tubing and four polishes of stainless steel (180, 320 and 500 grit, and electro-polish) were incorporated in the line.

The sections of tubing were joined with specially designed ferrules and gaskets. Ferrules were so made that they seated metal-to-metal with a thin gasket in between. This was desirable as it prevented the possibility of a slight separation of the joint should some unusual force be applied to the pipe.

Soft rubber, neoprene and Teflon gaskets were used. Pyrex sections were drawn together against a soft rubber gasket, and the stainless steel sections were joined alternately with a neoprene and then a Teflon gasket. By using a neoprene gasket at one end and a Teflon gasket at the other end of a section,

Received for publication March 8, 1951.

[1] This study was made possible by a scholarship granted the senior author by the Dairy Industries Supply Association.

each polish of the pipe was in contact with two kinds of gaskets. Examples of the fittings and gaskets used are shown in figure 1.

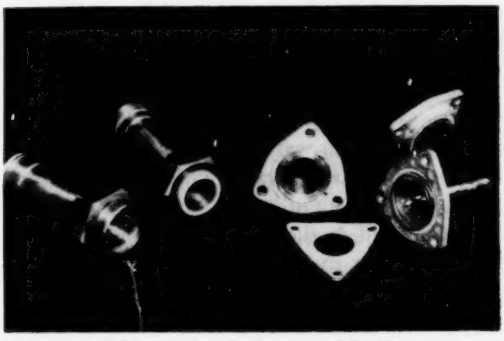


FIG. 1. Fittings (stainless steel and glass) used in the permanent pipe line.

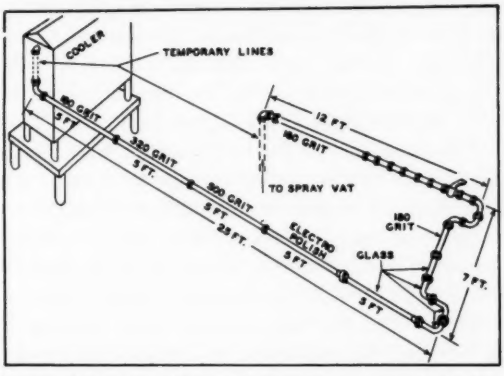


FIG. 2. Diagrammatic drawing of the permanent pipe line.

The pipe line (figure 2) was installed so that milk could be pumped into it either directly from the pasteurizing vat, as in the case of skimmilk, or from the homogenizer when homogenized products were processed. In the 49 ft. of pipe used, a pitch of 4 in. was incorporated to permit drainage.

Cleaning and sanitizing the permanent pipe line. The pipe line once assembled was never broken into for any reason other than making swab tests or plating gaskets. Washing and sanitizing were accomplished with a circulation system. The circuit was completed by connecting a hose between the outlet of the line and a 30-gal. movable supply tank at the inlet from which liquid was circulated by means of a 3,500 r.p.m. centrifugal pump. A velocity of 2.5 ft. per second was attained.

Upon completing the day's run of product,[2] the hose, surge tank and pump

[2] The total amount of product handled daily was in the order of 2,500 lb. and consisted of whole milk, skimmilk, chocolate milk or ice cream mix. Product temperature varied from 125 to 160° F.

were connected. Water at 100° F. was flushed through the pipe line until it appeared clear at the discharge end. Then acid cleaning solution was prepared in the surge tank by mixing 0.5 pt. of cleaner with 20 gal. of water. The temperature was raised to 130 to 140° F. by direct steam injection. The connecting hose was put in the tank and circulation begun. After 15 min., the acid solution was drained. An alkaline solution (prepared by adding 1 lb. detergent to 20 gal. of water) then was circulated at 160 to 170° F. for 15 min. The pipe line then was sanitized by circulating water at 180° F. for 5 min. To avoid formation of water stone, this water was treated with alkaline detergent (1 oz. per 20 gal. of water). Finally, the pipe line was allowed to drain until the following day, when it was re-sanitized in the same manner just prior to use. The acid cleaner contained 30 per cent hydroxyacetic acid, 20 per cent phosphoric acid and 5 per cent wetting agent. The alkaline cleaner contained 39 per cent sodium tripolyphosphate, 51 per cent metasilicate, 5 per cent wetting agent and 5 per cent soda ash.

Examination of the pipe line. Four methods of examination were employed in the evaluation of the sanitary condition of the pipe line. Milk samples were examined for "pick up" of bacteria. Milk samples were collected at the inlet and the outlet and examined for bacterial population. All samples were examined for mesophilic, psychrophilic and coliform bacteria. Measurements of pH also were made. The remainder of the samples then were placed in a 40° F. refrigerator and stored for 7 days. After storage they again were examined for bacterial population and pH to determine whether changes had occurred which could be traced to the pipe line. Swabs of each type of stainless steel, the pyrex glass and the gaskets were made at weekly intervals. These were examined for mesophilic bacteria. The third measure of efficiency was the direct plating of gaskets for mesophilic bacteria. The final test was the visual inspection of the pipe line.

Milk sampling technic. To enable the collection of milk samples in an aseptic manner, pet cocks were inserted in 6-in. sections of pipe at the inlet and outlet of the pipe line. These short sections were autoclaved before each experimental run. Milk samples were taken as the first milk entered and as it left the pipe line. After the vat was two-thirds empty, another set of samples was taken. Later in the study, samples also were taken from the vat.

Bacteriological methods. All plate counts were made using standard bacteriological procedures. Eight plates were prepared from each sample. Seven plates were poured with tryptone-glucose-extract (TGE) agar and one with violet red bile (VRB). Five of the TGE plates were incubated at 32° C. for 48 hr. to obtain the standard plate count. Psychrophilic bacteria were determined by incubating the other two TGE plates at 10° C. for 7 days. The VRB plate was incubated at 32° C. and read after 24 hr.

An average of TGE plates incubated at 32° C. was calculated and a standard deviation of the mean determined as described by Halvorson and Ziegler (5). By taking plus or minus two standard deviations of the mean, 96 per cent of the cases could be included and a range of the actual mean determined. If the

ranges did not overlap, it was considered that the counts represented different milk populations.

As a further indication of possible bacteriological activity, the pH of the stored milk samples was compared with the pH of the fresh milk samples. Measurements were made on a Beckman Research Model G pH meter.

The procedure for making the swab tests followed as closely as possible the recommendations of Tiedeman *et al.* (11). In making the tests, the pipe line first was broken. Then a swab was removed from the test tube, in which it had been sterilized, and immersed in the buffer solution, after which it was "wrung out" on the inside of the vial. The swab was applied to the pipe line surface using an alternating clockwise-counter-clockwise motion for four complete revolutions contacting approximately 14 in.^{2} of surface; the swab was reinserted in the vial and the stick broken off below the finger contact point. The vial was tightly stoppered and placed in a portable ice box. The gaskets were swabbed using a similar clockwise-counter-clockwise motion. One ml. of the swab solution was plated in standard TGE agar and incubated at 32° C. for 48 hr.

Gaskets, after being swabbed, were plated directly. The entire gasket was removed aseptically from the joint and dropped into a sterile petri dish. In the case of rubber gaskets between pyrex sections, only the inside $\frac{3}{8}$ in. of the gasket is in contact with the pipe, the rest being exposed to the outside air. Thus, it was necessary to cut away all but the inside $\frac{3}{8}$ in. before plating. This was done with shears which had been sterilized in the autoclave. As soon as gaskets had been dropped into the petri dishes, they were removed to the laboratory and plated.

Visual inspection of the pipe line. In this study, any visible build-up of soil was considered as undesirable. When the pipe line was broken for weekly swab tests, the sections were inspected for the presence of milk solids or other dairy soil. To facilitate this examination, five 8-in. sections of pipe were included in the line. One section was removed and carefully examined each week. A second piece was left intact for 2 wk. and then inspected. A third section was left 4 wk. and the fourth and fifth sections were untouched throughout the experimental period of 20 wk.

The sanitary pipes were examined by two methods. First, the section was removed from the line and held before a light. By sighting through it, an indication of brightness could be obtained. As a control, a similar section of equal age and use taken from a brush-washed line was held alongside for comparison.

The second method used was the flannel test. After sighting through the pipe, a clean, water-dampened white cotton flannel was rubbed vigorously inside the tube. The flannel then was examined for visible soil.

EXPERIMENTAL RESULTS

Bacteriological examination of fresh milk samples. Statistical analysis of the data obtained from the standard plate counts of 41 sets of samples of fresh milk shows little difference between the counts of the milk as it entered and as it left the pipe line. The counts of 27 of the 41 samples of the first milk through

the line are higher than the counts of the milk leaving the system (table 1, columns 2 & 3). This is further shown by the logarithmic averages (GM) of the counts. Moreover, when range is considered, in twelve instances the count of

TABLE 1

Standard plate counts of product samples taken from the experimental pipe line

Run	First product through the line			Last product through the line			Product processed
	Vat	Inlet	Outlet	Vat	Inlet	Outlet	
1		1,480	1,560		.....	.....	Skim
2		1,200	1,000		.....	.....	Skim
3		* 592	398		.....	.....	Chocolate
4		* 414	1,094		460	570	Chocolate
5		2,570	2,772		2,225	2,142	Homo
6		*1,178	988		985	760	Skim
7		2,786	2,944		220	206	Homo
8		2,900	3,707		810	728	Ice cream mix
9		*7,864	770		* 322	248	Homo
10		*8,730	3,028		462	438	Homo
11		445	465		430	395	Skim
12		*2,385	2,119		* 475	316	Chocolate
13		272	240		142	110	Skim
14		446	482		450	452	Skim
15		.....	.....		232	238	Skim
16		582	513		87	103	Ice cream mix
17		740	682		705	650	Homo
18		1,464	1,468		.....	.....	Homo
19		* 296	3,234		* 226	1,280	Homo
20		*1,984	1,252		2,057	2,052	Skim
21		1,614	2,042		1,410	1,370	Homo
22	1,622	1,412	805	1,326	756	844	Skim
23	1,470	*3,438	4,235	1,378	2,490	2,280	Homo
24	314	2,960	2,924	118	868	542	Homo
25	655	3,918	3,818	232	*1,220	735	Ice cream mix
26	185	.....	238	208	.....	200	Skim
27	292	*6,344	3,182	298	1,276	1,400	Homo
28	328	1,998	1,848	276	* 912	540	Ice cream mix
29	888	460	385	284	380	252	Homo
30	130	*4,628	2,620	55	672	700	Homo
31	262	2,432	865	200	302	340	Homo
32	200	3,288	2,975	128	668	542	Homo
33	158	*1,065	4,650	142	490	580	Ice cream mix
34	1,780	*6,125	2,418	1,938	2,610	2,355	Homo
35	150	*1,040	635	125	218	252	Homo
36	153	*8,975	538	298	462	455	Homo
37	148	175	190	125	168	258	Ice cream mix
38	270	* 322	165	37	* 100	220	Homo
39	283	443	260	88	307	98	Homo
40	200	* 285	322	145	* 303	198	Homo
41	680	1,105	1,092	.....	450	858	Homo
42	130	350	305	140	218	160	Homo
43	480	560	543	460	522	1,020	Homo
GM**		1,310	1,025		497	455	

* Significant difference between counts at inlet and outlet.
** Geometric Mean.

the milks entering the pipe line exceed the count of the milks leaving the line by a significant difference, whereas the count of the milks leaving exceed the count of those entering only five times. This relationship is even more evident when

considering the last milk through the line. This may be explained in part by contamination added by the homogenizer and the dilution within the pipe line. Whole milk passing through the system "picks up" organisms from the homogenizer. It is thought that most of these bacteria are washed out with the first milk, and as the initial portion passes through the pipe line, it becomes more diluted with the milk following until the pipe fills. This reasoning is supported by the drop in the count between the first and last milk through. Further, it is noted that vat counts are much lower than line sample counts on the first milk through. While it is impossible to know how much of the count is due to contamination and how much to the breaking up of bacterial clumps, it is evident that much of the increase resulted from bacterial pick up from the homogenizer, as vat counts and counts on the last milk through were much closer than vat counts and counts on the first milk through. From these data it may be concluded that the use of the permanent pipe line did not result in significant contamination of the pasteurized milk with mesophilic bacteria.

TABLE 2

Standard plate counts of psychrophilic bacteria of samples taken from experimental pipe line before and after storage at 40° F. for 7 d.

Run	First product through the line		Last product through the line	
	Fresh	Stored	Fresh	Stored
30	1,500	32	320	3,700
31	95	30,000+	10	10
32	16,000	30,000+	440	30,000+
33	1,700	30,000+	40	30,000+
34	500	30,000+	120	30,000+
35	40	70	75	50
36	250	5,800	190	3,100
37	15	110	15	1,400
38	180	30,000	30	15
39	55	31,000	10	15
40	55	1,900	45	200
41	310	1,200	30	280
42	55	78,000	55	210
43	25	4,000	55	9,000

With the exception of four cases, coliform counts remained negative throughout the experiment. In each of the four outbreaks, the source of the contamination was found to be the homogenizer packing which had become contaminated from a synthetic calf feed previously processed with the machine. This product contained quantities of lard and, while it was pasteurized, it left the gaskets in a condition which did not permit thorough sanitizing. By changing gaskets after each batch of calf feed, coliforms were eliminated in the experimental milks. At no time was the permanent pipe line ever found to contribute coliform bacteria.

In most cases, psychrophilic counts were so low on fresh milk that 30 colonies were not obtained on 1–to–10 dilutions. A comparison of the bacterial counts before and after storage at 40° F. for 7 days shows that the psychrophilic count increased during storage (table 2).

In the majority of cases the count of the milk samples held at 40° F. for 7 days showed the last milk through the pipe line to have a lower count than that of the first milk through (table 3). However, this does not reflect a true picture, because there is no way to tell how much the homogenizer contributed to these counts. The important fact is that on only 12 occasions did the count exceed 10,000 and only eight times did it exceed 30,000. On two occasions where counts were unusually high, it was found that on the weekend when the plant was shut down, the temperature had risen above 40° F. in the room where the samples were stored. Undoubtedly this had some effect on the numbers of bacteria in the milk.

TABLE 3

Standard plate counts of samples taken from the experimental pipe line and stored at 40° F. for 7 d.

Run	First product through the line	Last product through the line	Run	First product through the line	Last product through the line
1	1,000	1,000	23	2,400	1,600
2	1,400	300	24	TNTC	530
3	14,000	9,500	25	12,000	700
4	1,600	450	26	120	200
5	1,600	1,700	27	170	210
6	800	540	28	2,600	120
7	2,500	170	29	180	130
8	42,000	36,000	30	265,000	6,700
9	290	120	31	120	310
10	83,000	700	32	TNTC	440
11	430	190	33	1,800	480
12	3,900	460	34	12,000	6,600
13	340	110	35	260	80
14	280	350	36	5,300	260,000
15	150	120	37	5,900	990
16	120	130	38	7,600	30
17	1,100	600	39	28,600	70
18	.....	.....	40	1,700	160
19	5,500	1,100	41	1,700	990
20	500	480	42	80,000	290
21	1,500	2,300	43	3,800	4,900
22	450	430			

All samples after storage were palatable and only slight change in pH occurred. The data did not indicate that passage of the milk through the pipe line detracted to any appreciable extent from the storage qualities of the experimental milk samples held at 40° F. for 7 days.

The counts made on the swabs are given in table 4. Logarithmic averages (GM) show comparisons between the numbers of bacteria removed by swabbing weekly the same sections of pipe line. While no spot was found to be sterile on repeated examination, counts exceeding 100 occurred only seven times in 134 tests. These counts would be considered excellent when judged by the standards for the examination of restaurant utensils as recommended by Tiedeman *et al.* (11). On two occasions, high counts were incurred at points where a gasket had been pinched when the section was replaced after the previous swabbing. This

TABLE 4

Swab counts of stainless steel and pyrex glass sections of the experimental pipe line[a]

Swab no.	180 grit	500 grit	320 grit	Electro-polish	Glass	Glass	Glass	Glass	180 grit Ell	180 grit Ell
1	0	8	0	0	4	0	20	0	.....	.....
2	0	8	LEAK[b]	LEAK[b]	0	20	8	4	0	0
3	4	4	0	24	20	32	16	28	32	4
4	0	44	0	8	32	340	8	48	4	0
5	8	8	8	16	0	16	0	52	52	0
6	0	4	LEAK[b]	LEAK[b]	4	16	.....	.....	.....	.....
7	16	4	4	8	4	4	4	0	0	0
8	4	12	12	112	8	4	100	12	72	4
9	4	8	68	16	0	0	0	0	0	68
10	4	4	4	36	24	0	4	48	16	4
11	24	16	24	12	12	0	12	4	4	4
12	4	0	4	4	8	0	8	0	12	0
13	16	12	20	100	4	0	12	28	4	20
GM	5.6	7.0	5.5	9.8	5.4	4.4	7.6	7.2	6.8	3.2

[a] Swabs taken at weekly intervals immediately following sanitization.

[b] Leaky joint due to improper seating of gasket which resulted in numerous milk remnants being picked up by swab which caused extremely high count.

permitted a slight leak into the fitting, and although the heat of the sanitizing water penetrated the pipe, it apparently was not sufficient to kill the organisms in the entrapped milk. This demonstrates the necessity for maintaining tight-fitting joints.

There was little difference between the surface growth on the stainless steel and the glass, or on the different polishes of stainless steel. The highest counts on the stainless steel were obtained from the electro-polish section and the lowest from the 500 grit. However, this lower count is not important as all counts were low. While glass is smoother than stainless steel, counts were in the same order of magnitude and no difference in the bacterial growth could be attributed to this factor.

Swabs on the gaskets, while seldom sterile, never showed high count. Logarithmic averages of the counts taken over a 13-wk. period are as follows:

	GM	*Range*
Neoprene	13.0	0–48
Teflon	13.7	4–80
Rubber	20.5	4–400

These low counts are important, for if any spot on the line was contaminated, it would be expected to be the joints. Total counts obtained on plated gaskets were low. In no case did a gasket show more than 37 colonies on the surface which was in contact with the milk.

Visual inspection of the pipe line. At no time during the experimental period did the 8-in. sections which were examined at regular intervals show any accumulation of milk solids. When each was compared visually with the control (brush-washed section), no difference could be noted between the two. Flannel tests never revealed any evidence of milk solids.

After 5-mo. usage, the pipe line was completely dismantled and thoroughly inspected by the group who planned the project. Every section of pipe was as bright and clean as the original material with the exception of the section of electro-polish stainless steel. This section had become slightly coated with milk solids on the upper surface extending about 14 in. into the pipe from the end attached to the pyrex glass tubing. It was at this point that milk entered the tubing. The reason for this accumulation may be explained by the difference in diameters of the pipes. The glass tubing was 1.5 in. inside diameter, whereas the stainless steel tubing was 1.5 in. outside diameter; thus, the stainless steel had $\frac{1}{8}$ in. smaller diameter. This resulted in the rubber gasket extending past the opening of the pipe, creating a "blind" spot at the entrance. It was reasoned that this prevented the cleaning solution from contacting the upper surface of the pipe.

The gaskets also were inspected at the same time the pipe was dismantled. Neoprene and Teflon gaskets showed almost no signs of wear, but the rubber gaskets which had been in service throughout the 5-mo. period were cracked and disintegrated so that pieces easily could be scraped away with a knife.

SUMMARY

A permanent sanitary pipe line consisting of four polishes of stainless steel and pyrex glass was installed at the University Dairy. Study was made to determine if such a line could be satisfactorily cleaned by rapid circulation of acid and alkaline detergents and sanitized by the circulation of 180° F. water.

Milk samples were taken from the inlet and outlet of the pipe line and examined for mesophils, psychrophils and coliform organisms. Measurements were also made of the pH of the milk. Similar samples were stored at 40° F. for 7 days and again examined as before.

Sections of the tubing were swabbed regularly to determine the presence of mesophils remaining on the surface of the pipe line after sanitization.

Visual inspection of the line was made weekly. Comparison of sections which had been in service was made with a control consisting of a brush-washed section of stainless steel. A further test was made by vigorously rubbing a clean, white flannel within the pipe to pick up any loose deposits.

There was no measurable pick-up of organisms resulting from the use of the permanent pipe line. Samples of milk that had passed through the pipe line were held at 40° F. for 7 days. These samples showed no marked growth of mesophils and only slight increases in psychrophils. Only slight changes in pH occurred during the storage period. There were nearly as many changes to a higher pH as to a lower pH.

Swab counts on the pipe line were low. While sterility was not attained the results would be judged excellent by the standards recommended for restaurant utensils. Bacteriologically speaking there was no choice between the four polishes of stainless steel and the pyrex glass.

Under the conditions of this study, a permanently installed pipe line of either stainless steel or glass was found to be satisfactory from a sanitary point of view.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the following who aided in planning the study and evaluating the results obtained: H. Wainess, U. S. Dept. of Public Health; E. Huffer, Illinois State Dept. of Public Health; C. Weinreich and H. C. Lingle, Research Dept., Cherry-Burrell Corp.

REFERENCES

(1) Fay, A. C. The Use of Permanently Installed Glass Tubing in Milk Plants. Presented at the University of Illinois Market Milk Plant Operator's Conference. January 18, 1950.

(2) Fisker, A. N. Chemical Cleaning of the Pasteurization Plant. XIIth International Dairy Cong., 3: 3a–5: 319–324. 1949.

(3) Fleishman, F. F., Jr., White, J. C., and Holland, R. F. Glass Lines: Do A-1 Job; No Take Down to Clean. Food Ind., 22, 10: 1686–1689, 1821, 1823. 1950.

(4) Funke, A. H., Blizzard, N. J., and Blizzard, J. R. How Glass Piping is Used in Food Processing Plants. Food Ind., 10, 2: 77–79. 1944.

(5) Halvorson, H. O., and Ziegler, N. R. *Quantitative Bacteriology.* Burgess Pub. Co., Minneapolis, Minn. Pp. 14–15. 1938.

(6) Nelson, A. I. University of Illinois, Urbana. Personal communication. Feb., 1950.

(7) POSTHUMUS, G. Cleaning of Dairy Equipment and Control of the Efficiency of the Cleaning Process in Holland. XIIth International Dairy Cong., 3: 3a–5: 288–292. 1949.

(8) SHAKMAN, J. G. Pabst Brewing Co., Chicago. Personal communication. Aug., 1950.

(9) STEWART, A. P. Golden State Co., Ltd., San Francisco. Personal communication. Sept., 1950.

(10) STURM, A. W., WILSON, R. C., AND AHLSTROM, W. Cleaning of Milk Lines by Circulation. Panel Discussion Presented at State College of Agriculture, Pullman, Washington. March, 1950.

(11) TIEDEMAN, W. D., FUCHS, A. W., GUNDERSON, N. O., HUCKER, G. J., AND MALLMANN, W. L. Food Utensil Sanitation Proposed as Official Association Procedure. Am. J. of Pub. Health, 38(5): Part II, 68–70. 1948.

THE DEAERATION OF RAW WHOLE MILK BEFORE HEAT TREATMENT AS A FACTOR IN RETARDING THE DEVELOPMENT OF THE TALLOWY FLAVOR IN ITS DRIED PRODUCT

GEORGE R. GREENBANK AND PHILIP A. WRIGHT[1]

Bureau of Dairy Industry, Agricultural Research Administration, U. S. D. A., Washington, D. C.

The tallowy flavor is the principal type of spoilage which develops in stored dried whole milk. The tallowy odor is apparent when the container is newly opened and the flavor when the dry milk has been reconstituted to the liquid state. The development of this tallowy flavor never has been avoided successfully. However, its development may be greatly retarded by storing at low temperatures, packing in an atmosphere of inert gas such as nitrogen after removal of the air in the headspace of the container, high temperature preheating of the raw fluid milk and various other handling and manufacturing methods starting immediately after the milk is obtained from the cow.

Some years ago it was discovered in these laboratories that the high heat treatment of fluid milk, such as at 85° C. for 20 min., improved the keeping quality of the dried product (4). Heat treatment of milk at temperatures above 70° C. has been shown by several investigators (3, 5), to form reducing compounds such as the sulfhydryls. Greenbank (1) found that heating fluid milk at a temperature above 70° C. reduces the oxidation-reduction potential and thereby presumably retards oxidation. He postulates that these reducing compounds assist in the regeneration of the natural antioxidants in the dried milk.

It seems logical to assume that the greater the concentration of these reducing sulfur compounds the more efficiently the natural antioxidants in the dried milk will be regenerated. Since these sulfur compounds are easily oxidized, it seems logical also to assume that a decrease in the oxygen content of the fluid milk by deaeration before preheating will increase the concentration of the sulfhydryl groups, which in turn will regenerate the natural antioxidants and thereby retard the development of the tallowy flavor in the dried milk.

In order to obtain data in support of this last assumption, two series of experiments were carried out. One was on a laboratory scale, the other on a semi-works scale. In the laboratory series, deaerated and nondeaerated raw whole milks were heated at 85, 90 and 95° C. in sealed, full, baby-size cans and the oxidation-reduction potential determined at 5-min. intervals. Also, a set of control samples was heated in an open pan to simulate hotwell preheating. The values obtained are shown in figure 1. Data were obtained by heating the control samples at all three temperatures. However for the sake of clarity only the values of the control samples heated at 85° C. are shown.

The control data show the small decrease in E_h of milk heated under the usual procedures in which the milk contains dissolved air. The decrease in E_h of the deaerated milks was much greater than that of either the milk heated in the

Received for publication March 8, 1951.

[1] Deceased.

open pan or of the milk heated in a closed vessel without deaeration. Deaeration conserves the concentration of reducing compounds, presumably because there is less loss of reducing compounds by oxidation. The values obtained for the deaerated milk heated at 95° C. are lower than those for the milks heated at 85 and 90° C. (2). Harland *et al.* (3) obtained similar results on reconstituted dry skimmilk which had been preheated at 85 and 95° C. This may be due to a recombination at this higher temperature of the sulfhydryls with the protein.

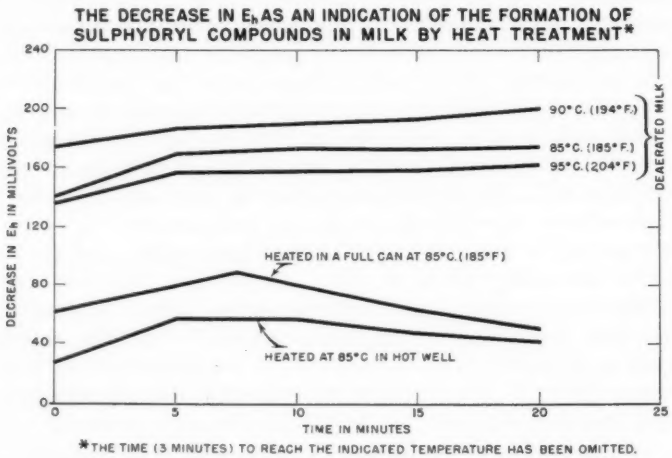


FIG. 1. The formation of reducing substances by heat treatment, in the presence and absence of air, as indicated by the decrease in E_h.

With the foregoing data in mind, dried whole milk was prepared on a semi-works scale. The milk was precondensed in a stainless steel vacuum pan and dried in a small, commercial-size Gray-Jensen dryer. The fluid milk for the control samples was preheated in an open kettle. The milk for the experimental samples was deaerated in the vacuum pan and preheated under partial vacuum in the vacuum pan for the same time and at the same temperature as the control samples. The only place where the deaerated milk came in contact with air was in the dryer. Here the temperature drop is so rapid and the time in the liquid state so short that in all probability oxidation is not appreciable.

Thirty-four pairs of dried milk samples were prepared by the methods described and their keeping quality determined after being packed in nitrogen and stored at 37° C., or in air and stored at 30° C. The number of days necessary for the dried milks to develop a flavor so tallowy as to render them inedible was a measure of the keeping quality. The average percentage increase in keeping quality for all the pairs of dried milks was of the same order as the average for those given in table 1. Table 1 gives the production, storage and keeping quality data for five representative pairs of dried milk samples. The dried milks prepared from raw milk which had been de-

TABLE 1

The effect of deaerating milk before heat treatment on the keeping quality of its dried product

Temp.	Time	Storage temp.	Packing	Keeping quality in days		Percentage increase in keeping quality
				Hotwell method	New method	
	(*min.*)					
175	30	30	Air	255	325	31.0
185	10	30	Air	126	168	33.0
160	20	37	Nitrogen	130	181	39.0
185	30	37	Nitrogen	84	180	118.0
185	20	37	Nitrogen	75	138	84.0

aerated before preheating have a longer keeping time than those prepared from raw milk without deaeration before preheating.

Harland, *et al.* (3) have published work which confirms the results shown in figure 1, both as to the concentration of the sulfhydryl groups and also their effect on the retardation of the tallowy flavor in stored milk powder. These reducing compounds may function in two ways in retarding the tallowy flavor: (a) Regeneration of the natural antioxidants. (b) After the dried milk is packed these sulfhydryls, which oxidize more rapidly than does the fat, may use up some of the residual oxygen in the can and so prevent it from combining with the fat to produce the tallowy flavor.

In other work (6) on the retardation of the tallowy flavor in which ascorbic acid was added to milk before drying, the authors found that if there were sufficient antioxidants in the milk powder to keep the apparent ascorbic acid content above a certain level, the dried milk would keep exceptionally well without the development of the tallowy flavor. This could be explained by assuming that the reducing compounds formed during storage may use up all the residual oxygen or more probably the sulfhydryl compounds formed during preheating assist in keeping the concentration of reducing compounds above the level where oxidation of the fat occurs (6).

SUMMARY

A greater decrease in oxidation-reduction potential and also a higher concentration of reducing compounds are found in milk deaerated before heat treatment than in milk heated without deaeration.

Deaeration before heat treatment of the raw fluid milk retards the development of the tallowy flavor in the dried product.

REFERENCES

(1) Greenbank, G. R. Variations in the Oxidation-reduction Potential as a Cause for the Oxidized Flavor in Milk. J. Dairy Sci., 23: 723–744. 1940.

(2) Greenbank, G. R., and Wright, P. A. The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. J. Dairy Sci., 31: 698–699. 1948.

(3) Harland, H. A., Coulter, S. T., and Jenness, R. Some Factors Influencing the Reducing Systems in Dry Whole Milk. J. Dairy Sci., 32: 334–344. 1949.

(4) HOLM, G. E., AND GREENBANK, G. R. The Keeping Quality of Butterfat with Special Reference to Milk Powder. Proc. World's Dairy Congress, **2**: 1253–1265. 1924.

(5) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of Heat Labile Sulfides of Milk. J. Dairy Sci., **26**: 689–703. 1943.

(6) WRIGHT, P. A., AND GREENBANK, G. R. The Effect of the Ascorbic Acid Content of Fluid Milk upon the Keeping Quality of its Dried Product. J. Dairy Sci., **32**: 644–648. 1949.

A COMPARISON OF PENICILLIN, STREPTOMYCIN AND SULFANILAMIDE FOR IMPROVING THE FERTILITY OF SEMEN FROM BULLS OF LOW FERTILITY[1]

JOHN O. ALMQUIST

Dairy Cattle Breeding Research Center, Pennsylvania Agricultural Experiment Station, State College

First evidence that antibiotics might be of value in improving the fertility of semen from bulls of low fertility was presented by Almquist (1) in 1948. Three out of five bulls showed large increases in fertility when penicillin was added to their semen. Because of the limited number of bulls in this trial, the present study was undertaken to obtain information on additional bulls used for artificial breeding which showed extremely low breeding efficiency. Streptomycin and sulfanilamide were tested in addition to penicillin, since fertility results involving relatively infertile bulls had not been reported previously with these antibacterial agents. Penicillin plus streptomycin also was included, since it has been shown (4) that the combination is much more effective for controlling bacterial growth than either antibiotic alone.

EXPERIMENTAL

Using the split-sample technique, a field trial was conducted in cooperation with the Western Pennsylvania Artificial Breeding Cooperative, Clarion, from January 2 to July 20, 1948. Only bulls with a breeding efficiency below 50 per cent (based on 90- to 120-day non-returns to first- and second-service cows) for at least 6 mo. preceding the experiment were used. Semen from seven low-fertility bulls (three Guernsey, three Holstein and one Jersey) was diluted with equal parts of egg yolk and 3.6 per cent sodium citrate dihydrate. The diluted semen then was divided into two portions, one of which served as the control. The other portion was treated with one of the following: (a) 1,000 units of penicillin per milliliter, (b) 1,000 γ of streptomycin per milliliter, (c) 1,000 units of penicillin plus 1,000 γ of streptomycin per milliliter of diluted semen or (d) 3 mg. of sulfanilamide per milliliter of diluter. Crystalline sodium penicillin G and streptomycin sulfate were used.

Inseminators were divided into two groups and each group received alternately treated and untreated samples from each bull. The experiment was designed so that four ejaculates per treatment or a total of 16 ejaculates was used from each bull. However, two of the seven bulls failed to complete the trial because of continued low fertility despite treatment of the semen.

RESULTS AND DISCUSSION

Table 1 presents the average fertility results based on 6-mo. non-returns for the seven problem bulls. A total of 3,920 first- and second-service cows were

Received for publication Mar. 10, 1951.

[1] Authorized for publication on Feb. 23, 1951, as paper no. 1660 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE 1

The effect of penicillin, streptomycin, penicillin plus streptomycin and sulfanilamide upon the fertility of semen from seven relatively infertile bulls

Treatment of diluted semen	No. 1st and 2nd services	Per cent non-returns after 6 mo.	Difference
Control	486	35	
Penicillin	475	54	+19**
Control	540	34	
Streptomycin	523	60	+26**
Control	525	36	
Penicillin plus streptomycin	519	59	+23**
Control	420	37	
Sulfanilamide	432	33	−4

** = Highly significant ($P = < 0.01$).

inseminated, of which 1,062 or 27 per cent represented second-service cows. Of this total, 1,971 cows were inseminated with control semen and 1,949 with treated semen.

Comparing each treated sample with its control, the effect of the individual treatments was determined by "Students'" method (8). The *t* values calculated for penicillin, streptomycin and penicillin plus streptomycin showed a highly significant improvement in fertility. The small decrease in fertility obtained with sulfanilamide was not mathematically significant. The *t* test also was used for comparing the average of the treated and untreated samples for each bull and results similar to those previously given were obtained. The differences obtained by subtracting the percentages of non-returns for the untreated portions from those for the treated portions of ejaculates were subjected to an analysis of variance. Only data from the five bulls completing the experiment were used. The analysis demonstrated that the differences in response between penicillin, streptomycin and penicillin plus streptomycin were not significant but that the response from sulfanilamide was significantly lower than that from any of the other treatments.

As mentioned previously, two of the seven bulls failed to complete the experiment. Only eight samples from each bull were treated, two with penicillin, two with streptomycin, two with the combination and two with sulfanilamide. One bull, a 3-yr.-old Jersey, showed no response, while the other, a 14-yr.-old Guernsey with relatively poor semen quality, showed some improvement with antibiotics but not with sulfanilamide. Based on 6-mo. non-returns for 111 services, the Jersey showed 32 per cent for the control semen and 31 per cent for the semen treated with antibiotics, while 113 services to the Guernsey showed 25 per cent for the control and 43 per cent for the treated. Even though the latter bull showed an improvement of 18 percentage units with antibiotics, the level of fertility reached (43 per cent) was not sufficient to warrant keeping him in active service.

These results confirm the report by Almquist (3) in which 1,000 units of penicillin per milliliter of diluted semen brought about large increases in fertility in three out of five relatively infertile bulls. In the present study, the same concentration of penicillin greatly improved fertility in five out of seven low-fertility bulls. Streptomycin alone or mixed with penicillin also exerted a similar beneficial effect. Although no significant difference was found between these treatments, diluters containing streptomycin gave slightly better results than penicillin. Sulfanilamide, on the other hand, failed to increase the fertility of low-fertility bulls.

Since publication of a preliminary report (2) on the experiment reported herein, Almquist and Prince (5) and Foote and Bratton (7) have presented additional evidence that bovine semen diluters containing penicillin and streptomycin, singly and in combination, could be expected to increase the fertility of relatively infertile bulls. However, the average fertility level of the untreated semen from the low fertility groups of bulls in these other reports was considerably higher than in the present study, while the degree of treatment response was much smaller. The latter is further substantiated by the recent findings of Easterbrooks *et al.* (6) using streptomycin alone in bovine semen. These workers found a definite inverse linear relationship between response and the initial level of fertility.

Although not effective in all relatively infertile bulls, the large degree of response obtained with a high proportion of the bulls tested appears to warrant the use of penicillin and streptomycin, alone or together, as a means of improving the fertility of semen from low-fertility bulls used for artificial breeding. The writer wishes to re-emphasize (3) that not all low-fertility bulls will respond to treatment of the semen with antibiotics; each problem bull will have to be tested by the trial and error method to determine the usefulness of the treatments suggested in this paper.

SUMMARY

Results obtained from seven bulls of low fertility on a split-sample experiment showed a highly significant average increase in fertility when yolk-citrate diluted semen was treated with either penicillin, streptomycin or penicillin plus streptomycin.

Based on 6-mo. non-returns, the improvement in fertility with penicillin, streptomycin and penicillin plus streptomycin amounted to 19, 26 and 23 more cows settled per 100 cows bred, respectively. However, the differences between the three treatments were not significant statistically.

The addition of sulfanilamide to yolk-citrate diluter did not increase breeding efficiency.

Two relatively infertile bulls failed to respond sufficiently to any of the treatments to continue in active service throughout the study.

The results of this experiment indicate that penicillin and streptomycin, either singly or together, offer a valuable means for improving the fertility of semen from certain bulls of low fertility in artificial breeding.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to G. W. Thompson and his staff of the Western Pennsylvania Artificial Breeding Cooperative for their assistance and cooperation. The penicillin and streptomycin were provided through the courtesy and cooperation of R. A. Patelski of Chas. Pfizer and Co., Inc., Brooklyn 6, N. Y.

REFERENCES

(1) ALMQUIST, J. O. The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. (Abs.) J. Dairy Sci., **31**: 681–682. 1948.

(2) ALMQUIST, J. O. A Comparison of Penicillin, Streptomycin and Sulfanilamide for Improving the Fertility of Semen from Relatively Infertile Bulls. (Abs.) J. Dairy Sci., **32**: 722. 1949.

(3) ALMQUIST, J. O. The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. J. Dairy Sci., **32**: 950–954. 1949.

(4) ALMQUIST, J. O., GLANTZ, P. J., AND SHAFFER, H. E. The Effect of a Combination of Penicillin and Streptomycin upon the Livability and Bacterial Content of Bovine Semen. J. Dairy Sci., **32**: 183–190. 1949.

(5) ALMQUIST, J. O., AND PRINCE, P. W. The Effect of a Combination of Penicillin, Streptomycin and Sulfanilamide upon the Fertility of Bull Semen. (Abs.) J. Dairy Sci., **33**: 393–394. 1950.

(6) EASTERBROOKS, H. L., HELLER, P., PLASTRIDGE, W. N., JUNGHERR, E. L., AND ELLIOTT, F. I. Fertility of Diluted Bull Semen Containing 100 Micrograms of Streptomycin per Milliliter. J. Dairy Sci., **33**: 737–740. 1950.

(7) FOOTE, R. H., AND BRATTON, R. W. The Fertility of Bovine Semen in Extenders Containing Sulfanilamide, Penicillin, Streptomycin and Polymyxin. J. Dairy Sci., **33**: 544–547. 1950.

(8) PATERSON, D. D. *Statistical Technique in Agricultural Research.* 1st ed. McGraw-Hill Book Co., Inc., New York, N. Y. pp. 20–22. 1939.

FORTY-SIXTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

P. R. ELLSWORTH, *Secretary-Treasurer*

The American Dairy Science Association assembled in the Alumni Memorial Auditorium at the University of Tennessee, Knoxville, Tennessee, on June 6th, at 9:30 a.m. Following the singing of the National Anthem, the Invocation and greetings from the University of Tennessee, Chairman, C. E. Wylie introduced Dr. R. B. Becker, President of the Association, who gave the following address.

THE PRESIDENT'S MESSAGE

Mr. Chairman, President C. E. Brehm, Dean J. H. McLeod, honored guests and members of the American Dairy Science Association, ladies and gentlemen.

With a feeling of gratitude, we acknowledge the hospitality of the University of Tennessee, its staff, the fine facilities on this campus and of the city of Knoxville. This is the 46th annual meeting of the American Dairy Science Association, and it is fitting that we meet in this part of the Old South, one of the cradles of dairy cattle interest in this country and the part of our land in which dairying is making rapid progress.

As evidence of this early interest, we witness the the "Book of Butter Tests of Jersey Cows," assembled and published by three pioneers of Tennessee dairying. Many older members know Sophie 19th of Hood Farm, a world record producer of butterfat and a great transmitting cow. Her ancestry came from Tennessee. The great bull Tormentor lived here and Hood Farm Pogis was bred at Spring Hill, Tennessee. The Jersey was the early dairy cow of the South. On this trip you will observe much cheese, condensed and evaporated milk and ice cream production. High quality market milk is produced where cows enjoy the sunshine and green nutritious pastures many months of the year. Tennessee was the first southern state to organize an artificial breeding association. So we are meeting in a long established and progressive dairy state.

I wish to discuss the American Dairy Science Association—what it is, what it does and the part members contribute to the dairy industry. Most services are directly by individuals, but some of far-reaching import stem from teamwork of specific Association committees of wide intersectional membership and by appointed representatives who speak and act for the best interest of industry and country.

R. B. BECKER

The American Dairy Science Association has passed through four stages of development. Professor Wilbur J. Frazier called a conference of dairy teachers and investigators in Agricultural Colleges and Departments of Agriculture in the United States and Canada in 1906. Practices and problems confronting teachers and the dairy industry were discussed. These stimulated further action by some upon returning home from the session. Subsequently, summer meetings were held at agricultural colleges accessible to the membership.

In the second phase, meetings were held in the fall simultaneously with the National Dairy Show. Staff members and coaches with dairy cattle and dairy products judging teams attended. Many of the second generation of our members obtained a vision of dairy possibilities at that time. Few could be away from campuses long during the scholastic year. The *Journal of Dairy Science* was established in 1918 as a bi-monthly technical journal, with publishing house responsible for its financial success. Officers were elected annually, the secretary serving one or two 1-yr. terms, until R. R. Graves became an "executive secretary"

when elected to successive terms.

At two sessions, it was proposed to return to summer meetings. Here more members could participate and families enjoy the associations. This culminated in the third phase when the first "new" summer session was held in 1927 at Michigan State College.

The fourth period began when the Association moved to own and publish the *Journal of Dairy Science*. To do so, law required that the Association incorporate legally, and vest responsibilities with an Executive Board. A new Constitution was adopted and certain business responsibilities undertaken. Officers were elected with overlapping tenures of office, the Secretary-Treasurer and Journal Editor being elected by the Executive Board. Certain committees were provided for in the Constitution, while others have a rotating or continuing tenure, giving greater continuity to their activities.

An annual meeting was omitted in 1945, by order of the Office of Defense Transportation. The Executive Board met for necessary association business.

What is the work of the American Dairy Science Association?

Educational programs stand out in which many members participate or attend, keeping up to date with developments in teaching, research and extension methods. This reflects immediately in the classroom, subsequent research, field activities and directly in commercial dairy work.

The *Journal of Dairy Science* expanded to monthly publication in 1934. It reaches every member, many subscribers and libraries. The Journal is used by advanced dairy students in most states and provinces and over the English-reading world. The Journal carries the highest class of advertising and reaches the quality reading public in the entire dairy field.

Few know of our participation on the National Research Council on the Committee for Biology and Agriculture, and that, hence, several council activities bear on dairy problems in broad aspects. The Committee on Brucellosis instigated a second National Research Council Committee under Dr. W. W. Spink, which discovered a cure for undulant fever. Another National Research Council Committee now active, will affect future sanitary codes in milk production and processing.

During World War II, some would dictate how much scarce protein feed a cow could have, and expected her to continue making milk without regard to character of the food. The National Research Council Committee on Animal Nutrition corrected that philosophy. A volunteer five-man committee of the American Dairy Science Association obtained allocation of urea for ruminant feeding, as Germany had used it in World War I. This movement was not without opposition.

How many recall that 4-H Club heifers once were owned by banks and business men? After 1-yr. care, all animals were sold at public auction. Dr. C. H. Eckles, as President, appointed a Calf Club committee which proposed this be changed to a breeding project with club members owning their heifers. Many former Club members are leading breeders of dairy cattle.

The original plan for Cow Testing Associations was proposed in Denmark by Mrs. Frederick Hansen in 1895. Hjalmer Rabild established one in Michigan in 1905. Extension dairymen grasped the plan, and through combined activities of Extension Section committees the Dairy Herd Improvement Associations have spread as instruments of dairy progress. Bull proving and calf identification programs were sponsored jointly with the Bureau of Dairy Industry. Great progress stems from this original project.

The Breeds Relations Committee unified supervision of official production records. They worked with breed associations and the Purebred Dairy Cattle Association to unify and improve activities dealing with purebred dairy cattle. Curricula and teaching methods have come up for periodic review. Professor H. P. Davis and a very wide committee are at work again on bringing curricula up-to-date with a rapidly moving industry.

Research methods have come in for a full measure of attention by committees of the Manufactures and Production Sections—Babcock test, bacteriological methods, butter committee, measuring value of pastures, methods of proving sires and dams, and many others. Some committees coordinate with similar committees of other scientific or regulatory organization. Some are joint inter-sectional committees such as the "Antibiotics Committee" established in 1950. The rapid strides in Artificial Breeding Associations have been possible because of foundations laid by several committees.

Committees are not an obligation. They widen services through coordinated teamwork. Some may shun participation, but the busiest men regard committee planning as a means of soliciting teamwork among leaders for more efficient future action. New committees are established as needs arise and disbanded when their goals are achieved.

I wish to deal with a third subject which has been given attention this year.

During World Wars I and II, this country gave little or no attention to other than war activities and urgent food production. Manpower in some fields was overlooked in favor of war emergencies. Drafts were made of men sometimes with little consideration to their loss from essential fields,

or the services to which they would be placed. Training of replacements in technical and research fields was neglected or totally disregarded, with war emergency as the major consideration. Some highly trained specialists were drafted as privates to services that could have been filled by persons with a grade or high school education. No provision was made to replace them.

Agriculture and agricultural research are essential to production of that first essential munition—food. How old is the axiom, that "The Army travels on its stomach"? Many present can speak first hand of importance of food to men in uniform. Yet the attitude of draft authorities placed the large burden ot proof on the draftee or his employer, instead of considering the vital part of specially skilled labor in efficiency of food production to our national welfare. A local draft board even has questioned whether or not one engaged in producer-distributor dairying was engaged in agriculture. Educational leaders called some of these oversights to the attention of national authorities, and partial consideration has been given to the problem. There is need for wide inter-organizational study of this problem and improvement of the national program that will place more decision in the hands of responsible institutional hands as to where a man can render the greatest service to national welfare.

The profession of farming and dairying has become increasingly the task of skilled mechanics and trained men that cannot be replaced on short notice from the ranks of labor. The skilled man in mechanized agriculture produces more food by far than the good farmer did under earlier practices. The same importance should be attached to their skilled services in food production as is given to the man producing implements of war. Neither product has full value without the other in times of national emergency. There is need for this phase to be given further consideration by the National Research Council and their coordinated organization, the National Security Resources Board, following somewhat a production index devised in New York state.

Use is being made of National Research Council committees now with regard to materials such as cobalt, essential alike in vitamin B_{12} and other nutritional and human medical uses and in production of certain highly important alloys. Another synthetic vitamin, niacin, is on the present critical list. As time goes on, other essentials may become in critical supply. These situations require alertness that food production be not impeded through unbalanced actions in times of preparedness. Wide understanding and broad viewpoints are essential to all concerned. It is in such actions that there is strength in democracy.

* * * *

Chairman Wylie then introduced Dr. Harry A. Curtis, Director, Tennessee Valley Authority, who spoke on "Grasslands and Hydroelectric Power".

Dr. Paul B. Pearson, Chief, Biology Branch, Atomic Energy Commission spoke on the "Impact of Atomic Energy on Agriculture."

The opening session adjourned at the conclusion of Dr. Pearson's address.

BUSINESS MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Knoxville, Tennessee, June 8, 1951

President Becker called the meeting to order at 3:00 p.m. in the auditorium of Ferris Hall. There were 100 members present.

REPORT OF THE EXTENSION SECTION

The extension section meeting was called to order at 1:30 p.m. by Chairman Raymond Albrectsen in Room 203 Physics and Geology Building.

After a brief welcome and discussion of the program, the group voted to dispense with the readings of the minutes of the previous session.

The Chairman appointed the following nominating committee: Gerald Heebink, West Virginia; Floyd Arnold, Iowa; E. J. Perry, New Jersey.

C. W. Reaves of Florida made a brief report concerning the resolutions committee.

R. Albrectson next initated the program by introducing Stanley Gaunt, Massachusetts, who as Chairman of the Teaching and Methods Committee presented their program. W. A. Dodge, Vermont, presented an excellent demonstration on mastitis prevention, using "Susie" to put across the message in a humorous skit.

G. F. Johnson, Visual Aid Specialist of the Pennsylvania State College, presented a discussion of and equipment for visual aids for extension men.

C. Y. Cannon of Iowa presented a television movie with lecture as part of Iowa's exhibit. E. J. Perry showed slides on farm management factors as part of New Jersey's exhibit. The group then adjourned to Room 210 for the other exhibits.

Twelve states had exhibits. These included, beside visual aids, dairy records, circular letters, pay check stuffers, roughage data, stalls and mechanical cows. After discussion of these exhibits, the meeting adjourned at 4:30 p.m. The attendance at this session was 69.

The extension meeting reconvened at 9:05 a.m. on June 7th, 1951. Chairman Albrectsen introduced M. E. Senger, Maryland, and D. E. Voelker, Iowa, who presented papers relating

to DHIA work. D. E. Voelker presented a panel on DHIA composed of Connelly of Virginia, Kendrick of Washington, D. C., Jim Burke of New York, Stinelt of Oklahoma, and Gilmore of Pennsylvania. Voelker then presented the report of the Dairy Record Committee which was voted on and adopted. Meeting adjourned 12:05 p.m.

The section reconvened in a joint session with the Production Section at 1:40 p.m. at the Auditorium of Alumni Memorial Hall. L. O. Gilmore opened the meeting and presented Earl Weaver, Michigan, who discussed the Dairy Shrine Club.

Joint Committee Reports then were given, voted upon and adopted. The report of the Breeds Relations Committee was given by E. C. Scheidenhelm. The report of the Dairy Cattle Health Committee was presented by William Knox. The Dairy Cattle Breeding report was discussed by Joe Taylor. The Type Report was read by George Hyatt, Jr., because of the absence of the committee chairman. The report of the Dairy Cattle Association was presented by Fred Idtse. The report of antibiotics was presented by W. A. Krienke.

L.O. Gilmore presented his Co-chairman, Ray Albrectsen, who then presented the panel members for the Symposium on The Role of Roughage in Milk Production. The panel members were J. B. Kitchen, Jr., New Jersey, W. T. Crandall, Cornell, L. A. Moore, Bureau of Dairy Industry, C. F. Huffman, Michigan State College, and F. B. Morrison, Cornell.

The section adjourned at 5:15 p.m.

Chairman Albrectsen opened the meeting at 9:06 a.m. He then turned the meeting over to the Vice-chairman, Ramer Leighton of Minnesota.

Two papers on 4-H Club work and three papers on Dairy Cattle Breeding were presented and discussed.

An acknowledgment of the first Extension Award winner in our midst was tendered E. J. Perry of New Jersey.

Adjournment for lunch at 11:50 a.m.

Meeting reconvened at 1:04 p.m. Two papers on artificial breeding were presented.

The Extension Section annual business meeting was held next. Committee reports were given, discussed and adopted.

Stanley Gaunt was elected as Secretary of the section for 1952. The officers of the Extension section for 1952 will be: Ramer Leighton, Chairman; I. E. Parkin, Vice-chairman; Stanley Gaunt, Secretary.

Respectfully submitted, Raymond Albrectsen, Chairman; R. D. Leighton, Vice-chairman; I. E. Parkin, Secretary.

Upon motion duly seconded, the report was accepted.

REPORT OF THE MANUFACTURING SECTION

The Manufacturing Section held six half-day sessions during which the contributed papers as listed in the official program of the Association meeting were presented. In addition, two half-day sessions were devoted to symposia, the first of these being on "Milk Fat," under the leadership of J. H. Hetrick, and the second on "Training Students at the College Level for the Dairy Industry," under the leadership of O. F. Garrett. The speakers in the two symposia presented papers by invitation.

The business meetings of the section were held on Thursday, June 7, at 11:15 p.m. and on Friday, June 8 at 11:15 p.m., with Chairman J. H. Hetrick presiding.

The report of the Nominating Committee, I. A. Gould, Chairman, presenting a slate of officers for the coming year, was duly accepted.

Reports of the following committees were read and accepted as read:

(1) Milk and Cream. D. V. Josephson, Chairman Subcommittees:
   (a) Standardization of the Babcock Test. E. O. Herreid, Chairman
   (b) Uniform Procedures for Making Acidity Determinations of Fluid Dairy Products. J. G. Leeder, Chairman
(2) Standardizing Methods for Conducting all Phases of the Babcock Test. G. H. Wilster, Chairman
(3) Standardization of Alkali Tests and Method of Reporting Results. D. H. Jacobsen, Chairman
(4) Judging of Dairy Products. G. M. Trout, Chairman
(5) Evaluation of Methods for Determining the Activity of Cheese Cultures. H. C. Olson, Chairman
(6) Nomenclature and Methodology of Milk Proteins. A. M. Swanson, Chairman
(7) Standing Committee on Antibiotics in Milk. W. A. Krienke, Chairman

The report of the committee on Butter was read by G. H. Wilster, Chairman. Upon motion duly seconded, the section voted to amend the report as follows: On page 2 of the report, under section "B—Method of Attack," paragraph (a) was amended to read as follows: ". . . All states should establish laws and/or regulations governing minimum sanitary methods of producing and handling milk and cream." Upon motion duly seconded, the section voted to accept the amended report of the Committee on Butter with the stipulation that the report be withheld from publication.

Upon motion duly seconded, the section ap-

proved appointment of the following committees to serve for the ensuing year:

(1) Uniform Procedures for Making Acidity Determinations of Fluid Dairy Products.
(2) Standardization of the Babcock Test.
(3) Standardization of Methods for Conducting all Phases of the Babcock Test.
(4) Standardization of Alkali Tests and Method of Reporting Results.
(5) Judging of Dairy Products.
(6) Evaluation of Methods for Determining the Activity of Cheese Cultures.
(7) Nomenclature and Methodology of Milk Proteins.
(8) Butter.
(9) Study of the Standard Plate Count with Particular Reference to Temperatures of Incubation, this committee to work with the American Public Health Association.

Upon motion duly seconded, the section voted to refer certain projects proposed in the report of the Committee on Milk and Cream to the following committees:

(1) Detergent methods for butterfat testing, to the Committee on Standardization of Methods for Conducting all Phases of the Babcock Test.
(2) Detection of antibiotics in milk, to the Standing Committee on Antibiotics in Milk.
(3) Critical review of the acidity test in milk and dairy products with particular reference to the indicator used, to the Committee on Uniform Procedures for Making Acidity Determinations of Fluid Dairy Products.

A motion was made and seconded that a committee be appointed to investigate the possibility of the section sponsoring the publication of a book on the chemistry of milk. Motion failed to carry.

Upon motion duly seconded, the following resolution was passed:

Whereas: There is an apparent and urgent need for a new and up-to-date book on the chemistry of milk, and

Whereas: Several eminently qualified persons have under consideration the writing and publication of a book on the chemistry of milk,

Therefore, be it Resolved: That the Manufacturing Section of the American Dairy Science Association encourage the publication of a book on milk chemistry and lend its support to those qualified persons who undertake the writing and publication of said book.

The following officers were elected to serve for the ensuing year: A. J. Morris, Secretary; O. F. Garett, Vice-chairman; E. L. Jack, Chairman.

Respectfully submitted, J. H. Hetrick, Chairman; E. L. Jack, Vice-chairman; O. F. Garrett, Secretary.

Upon motion duly seconded, the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held four sessions for presentation of technical papers, at which 88 papers were presented. During each session, two sections were run concurrently, with Chairman L. O. Gilmore and Vice-chairman N. N. Allen presiding. The authors of the papers are to be commended for having their material well prepared and for keeping well within the prescribed time limits.

On June 7 one intersectional session was held with the Extension Section, starting at 1:40 p.m. L. O. Gilmore presided. Dr. Earl Weaver of Michigan State College was introduced and discussed the Dairy Shrine Club.

The following joint committee reports were read and adopted:

Breeds Relations Committee, read by E. C. Scheidenhelm.
Dairy Cattle Health Committee, read by W. D. Knox, Chairman.
Type Committee, read by George Hyatt in absence of the committee members.
Dairy Cattle Breeding Committee, read by Joe Taylor, Chairman.
Purebred Dairy Cattle Association, read by F. S. Idtse, Secretary.
Antibiotics, read by W. A. Krienke, Chairman.

Following the joint committee reports, a symposium on the Role of Roughage in Milk Production was presented by the following panel: J. B. Kitchen, Jr., Rutgers University; W. T. Crandall, Cornell; L. A. Moore, Bureau of Dairy Industry; C. T. Huffman, Michigan State College; F. B. Morrison, Cornell.

Raymond Albrectsen presided at the symposium.

The joint business session of the production and extension sections adjourned at 5:10 p.m.

The Business meeting of Production Section was called to order at 11:30 a.m., June 8, 1951, by L. O. Gilmore, Chairman.

A report of the Dairy Cattle Judging Committee was given by George Trimberger, Chairman of this committee. It was moved by George Trimberger and seconded by I. W. Rupel that the report be approved. Motion passed. Moved by Rupel and seconded by Cannon that the Dairy Cattle Judging committee be sustained in its refusal to admit Louisiana Polytechnical Institute at Ruston for competition in the National Intercollegiate Judging Contest and that they be

advised why this action was taken. Motion passed.

A report of the Pasture Investigation Technique Committee was given by R. H. Lush. Moved by Lush and seconded by Seath that report be accepted. Motion passed.

Dwight Seath presented the report of the nominating committee in the absence of the chairman J. B. Fitch. The other member of this committee was A. O. Shaw. In keeping with the policy of recent years, N. N. Allen, present Vice-chairman, was nominated for Chairman, and George Hyatt, Jr., present Secretary, was nominated for Vice-chairman. The names of P. L. Kelly and Tom Mead were submitted for Secretary. The report of the nominating committee was accepted and the Chairman and Vice-chairman elected by acclamation. As a result of a ballot, P. L. Kelly of the University of Nebraska was elected Secretary of the Production Section.

A. A. Borland, Chairman of the Resolutions Committee, read his report and moved its adoption. It was seconded by K. Morrow. After some discussion by Wallace, Knox, F. B. Morrison and Espe relative to the report, and after revisions and deletion of certain parts of Section 6, the committee's report was approved.

Chairman L. O. Gilmore then called for new business. Gilmore discussed the possibility of mailing out mimeographed committee reports presented at the Production Sessions to Production workers at all institutions as is already done by the Extension Section, so that each and every one would have a copy of this material. A motion was made by Hyatt and seconded by Ely that this practice be followed at future meetings. Motion passed.

The following motion presented by George Hyatt was moved by F. B. Morrison and seconded by A. A. Borland to be passed on to the Journal Management Committee:

"Since the publication of the Journal of Dairy Science is an important function by which The American Dairy Science Association makes the results of dairy research and study available to the public, it is requested that the Journal Management Committee make every effort to have articles reported in such language as to be of as much value as possible to the majority of its readers and not just to those especially trained in scientific terms and statistical methods of research. There is a sizeable group of subscribers and potential subscribers to the Journal who can benefit from the reports in the Journal. Men in Extension, in commercial work, and in educational work with commercial people will benefit more if articles are written in such a language that they will be reasonably understandable to the non-technical professional men without losing their scientific value."

Motion was adopted.

Hyatt suggested that in the future, if possible, committee reports should be in the hands of the voting membership sometime prior to the annual meeting. A great deal of meaty material is contained in each of the committee reports and if the membership is not thoroughly acquainted with this material prior to the time of the meeting, they cannot vote on their adoption or rejection intelligently.

Chairman Gilmore expressed his appreciation to the University of Tennessee, particularly to all members of the Dairy Staff at the University of Tennessee, to all committee members and to the Section Vice-chairman and Secretary for their help in making this meeting a success.

Meeting adjourned 12:20 p.m.

Respectfully submitted, L. O. Gilmore; N. N. Allen; George Hyatt, Jr.

Upon motion duly seconded, the report was accepted.

REPORT OF THE NATIONAL RESEARCH COUNCIL

Division of Biology and Agriculture

One of the important functions of the Division of Biology and Agriculture is to promote progress in Agriculture. To supervise this, an Agricultural Board within the Division was organized in 1944 but because of the difficulty of finding financial support for its work it became inactive. Its need was felt to be so important that its reorganization was completed during the past year. Its membership now includes representation from the fields of agronomy, animal science, entomology, horticulture, plant pathology, range management, agricultural economics and industrial engineering.

This board now supervises the activities of the committee on animal nutrition, committee on feed composition, committee on animal health, committee on milk production, distribution and quality, committee on public health aspects of brucellosis and committee on veterinary services. Most of these committees have sub-committees working on special phases of their fields. It seems to be much easier to get financial support from industry to carry on this special work than to get finances to operate the activities of the Agricultural Board itself.

During the past year, 41 applications for research grants were received. After screening, 14 of these were recommended by the Division of Biology and Agriculture, three of which were accepted and provided with National Research Council funds. The others then were recom-

mended to the Atomic Energy Commission for help. One hundred and thirty applicants for Fulbright fellowships were screened to a recommended 32. There are now approximately 1,200 people abroad under the Fulbright act.

A Committee has been organized to preserve the many native strains of maize which are in danger of being lost because of the wide spread introduction of hybrids into many of the Latin-American countries. These native strains may have many characters that could be very useful in future breeding work.

The Division has clarified the relationship of scientifically trained men with the Armed Services. As never before, the services are fitting these men into places where they can make full use of their specialties. Recognition of the need for training the more capable high school and college students has resulted in modification of induction rules to keep them in school. It should be emphasized that these students are used as part of the advancement of the country just as much as the men in the army. They are not kept in school for the good of the colleges but for the country's need.

Through the army, navy and public health service, funds have been provided to the Division to gather and organize data pertaining to chemicals and biological materials. The data as collected are being codified and placed on IBM cards so that information concerning any chemical or biological activity can be sorted quickly for use by interested research workers.

Progress on the Handbook of Biological Data has reached the point where the tables on blood have been completed and the collection of tables on nutrition are well along.

Application for membership in the Division was received from the Society of Systemic Zoology but action on it was deferred for 1 yr. The Division now has 30 member societies which broadly represent its fields of activity.

Respectfully submitted, C. Y. CANNON

Upon motion duly seconded, the report was accepted.

EDITOR'S REPORT

Volume XXXIII printed during 1950 contained 1,154 pages other than advertising, 173 pages less than printed in 1949. A portion of this decrease is due to the fact that less than three pages were required to print the list of new members, whereas 39 pages had been required to print the complete membership list the preceding year. The number of abstracts in the abstract section decreased form 929 to 867 and only 161 pages were required to print them, whereas 186 pages were required in 1949. During 1950, 107 manuscripts requiring 803 pages and two production reviews requiring 49 pages were printed. Both number of manuscripts and number of pages represent appreciable drops from the preceding year. Another shift is apparent in that the proportion of production papers increased, 69 production papers and 38 manufacturing papers having been printed. Twelve manuscripts were rejected or withdrawn in 1950. An unusually large number (68) of manuscripts in various stages of preparation for publication was carried over in 1951, largely because greater numbers than usual were received in the last few months of 1950 and had not cleared review and editorial channels by the end of the year. This influx is reflected in the situation that 90 manuscripts will appear in the first seven issues in 1951, whereas 57 appeared during a similar period in 1950.

During the last several months the receipts of abstracts have been lower than in recent years. Only 339 abstracts will appear in the first six issues for 1951, whereas 459 were published during the same period of 1950. A saving in the costs of printing abstracts was initiated with January, 1951, issue by reducing slightly the space between lines, between title and body of abstract and between abstracts. The size of type used for some headings also was reduced. Certain decisions relative to the future of the abstract section are under discussion by the Journal Management Committee. The high cost in both money and effort of maintaining the abstract section make it imperative that there be sufficient evidence of value to the membership if this area of activity is either to continue as at present or be expanded.

Appreciation is expressed to all who have helped to do the work necessary for continuance of the Journal. The constructive and helpful attitude of those who have assisted in the review of papers, and thus must remain anonymous, is worthy of special comment and thanks. Our rejection rate is low, compared to many journals, probably in considerable part because the salvage rate on manuscripts not originally submitted in completely acceptable form is high. Critical review and constructive suggestions by reviewers are major factors in the final quality of many published manuscripts.

The editor wishes to express his appreciation to I. A. Gould and I. W. Rupel who are retiring as Associate Editors of the Journal, having completed their terms of office. G. H. Wise has completed his term as a member of the Journal Management Committee and his counsel in this capacity will be missed.

The editor regrets that he was unable to complete the preparation of the indices early enough to avoid delay in publication of the December, 1950, issue. The combination of unavoidable events which was responsible should not occur again and some assistance on this task also has been obtained.

Respectfully submitted, F. E. NELSON, *Editor.*

Upon motion duly seconded, the report was accepted.

SECRETARY–TREASURER'S REPORT

Membership

The following is a summary of our gains and losses for 1950:

Membership, December 31, 1949		1725
Gains:		
New Members	98	
Former Student Affiliates ..	20	
Total Gain	118	
Losses:		
Members resigned	22	
Members delinquent	112	
Members deceased	6	
Total Loss	140	
Net Membership Loss		22
Membership, December 31, 1950		1703

It might prove of interest to know the distribution of our membership:

Ala.	11	Ia.	39	Nev.	2	S.D.	9
Ariz.	10	Kan.	16	N.H.	10	Tenn.	22
Ark.	9	Ky.	19	N.J.	39	Texas	27
Calif.	82	La.	19	N.Mex.	3	Utah	13
Colo.	14	Maine	4	N.Y.	165	Vt.	14
Conn.	28	Md.	46	N.Car.	22	Va.	29
Del.	2	Mass.	39	N.D.	7	Wash.	36
D.C.	31	Mich.	49	Ohio	136	W.Va.	12
Fla.	31	Minn.	59	Okla.	17	Wisc.	111
Ga.	25	Miss.	10	Ore.	23	Wyo.	6
Idaho	11	Mo.	45	Pa.	104	Alaska	1
Ill.	165	Mont.	11	R.I.	4	Canada	32
Ind.	39	Nebr.	15	S.Car.	15	Foreign	15

New members for 1950 came from the following states and countries:

Ark.	1	Ia.	3	Nev.	1	S.C.	1
Calif.	5	Kan.	2	N.H.	1	Tenn.	2
Colo.	2	Ky.	1	N.Y.	16	Utah	1
Fla.	5	La.	1	N.C.	2	Vt.	2
Ga.	3	Md.	4	Ohio	8	Va.	4
Conn.	5	Mass.	3	Ore.	3	Wash.	2
Idaho	1	Mich.	5	N.J.	1	W.Va.	1
Ind.	1	Minn.	1	Pa.	6	Wisc.	7
Ill.	7	Mo.	3	R.I.	1	Foreign	6

JOURNAL CIRCULATION.

As might be expected in a year of increased membership dues and subscription rates, the circulation of the Journal fell to 3,679, a loss of 351 copies per month. There are still many libraries in the country who are not subscribing to the Journal. It does not seem unreasonable to state that the Journal should be part of every library both in educational and industrial circles. If your local library does not subscribe, perhaps the value of the Journal should be called to the librarian's attention. Many dairy industry men are not members of the Association, and there are numerous men in the Universities and Colleges who are not members. Some real effort expanded by everyone to obtain new members can overcome the drop in circulation most speedily.

STUDENT AFFILIATES.

During 1950 there were 750 student affiliate members of the Association. This compares with 859 for 1949.

The following schools have active Student Affiliate Chapters:

Alabama Poly. Inst.	Cornell Univ.
Univ. of Florida	Okla. A & M College
Univ. of Georgia	Pennsylvania State
Univ. of Kentucky	Clemson
Univ. of Massachusetts	Univ. of Tennessee
Michigan State College	Texas Tech.
Univ. of Minnesota	Va. Poly. Inst.
Univ. of Missouri	State College of Wash.
Montana State College	W. Virginia Univ.
Univ. of Nebraska	Ohio State Univ.
Rutgers	

ASSOCIATION FINANCE.

The Association operated at a net loss of $1,128.53 for 1950. This is a decrease of $5,666.31 over the previous year's loss. This improvement in financial condition may be accounted for by the increase in dues and subscription rates and by a decrease in Journal costs.

Income for the year increased $2,194.66 and operating expenses decreased $3,867.40 of which $3,805.23 can be accounted for by decreases in the cost of publishing the Journal and supplying reprints. Operation of the Association, not counting publication of the Journal, cost $9,011.32.

Four U. S. Government Bonds (Series "D") totaling $4,000 matured this year. Of this amount, $3,700 was reinvested in five Series "F" bonds. The balance was placed in the checking account.

For the year 1951, with increased advertising rates and further increased membership dues in effect, the outlook looks quite favorable. This, however, does not preclude the need for every member to obtain a new member for 1951.

Total income for 1950 was $29,635.87 which is the second highest income for any year of our organization's history, The 1948 income was $5.71 higher. Our net worth at the end of 1950 was $28,113.47. We had an investment in government bonds of $31,610.00. Our total assets were $43,750.29. A complete report of the Certified Public Accountant was sent to each Officer and Director in May.

The Secretary wishes to thank the Officers, Directors and other members of the Association for their splendid cooperation during the past year.

Respectfully submitted, P. R. ELLSWORTH, *Secretary-Treasurer*

Upon motion duly seconded, the report was accepted.

AUDITING COMMITTEE REPORT

To the Executive Board and Members of
The American Dairy Science Association

Gentlemen:

On May 24, 1951, Mr. Walter C. Burnham, a Certified Public Accountant met with the Auditing Committee of The American Dairy Science Association. At that time, Mr. Burnham's report of his audit of the Association's business for 1950 was considered.

Mr. Burnham has made a thorough examination of the records. He has checked the bank statements and examined all the U. S. Government Bonds. Mr. Burnham has check-tested the inventory of Journals and Twenty-Year Indices to assure accuracy of the physical inventory.

The Auditing Committee is satisfied that the financial statement for the year 1950 is correct. The committee wishes to commend Mr. Burnham, the auditor, for his fine work and excellent report. We recommend that the financial statement be accepted by the Executive Board and the members of the American Dairy Science Association.

Respectfully submitted, FORDYCE ELY; L. H. BURGWALD; S. M. SALISBURY, *Chairman*

Upon motion duly seconded, the report was accepted.

REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

Decisional action taken by the Journal Management Committee during the past year included:

1. Granting permission to Bailliere, Tindall and Cox, the British agents for the JOURNAL OF DAIRY SCIENCE, to authorize, at their discretion, photostatic reproduction of articles published in the JOURNAL, providing that copies of letters of authorization be filed with the Secretary of the American Dairy Science Association.

2. Recommending the appointment of J. H. Hetrick and Vearl Smith as Associate Editors to succeed I. A. Gould and I. W. Rupel, in accordance with the established rotational procedure.

3. Reaffirming the policy of publishing in the Journal only releases relating to the Association activities.

4. Authorizing the following measures to improve the financial status of the Journal.

a. Revision of list prices of back volumes and numbers of the Journal. Prices were increased, but a differential rate was made on the basis of Association membership.

b. Increase in advertising rates, effective January 1, 1951.

c. Alteration of organization of abstract heading to conserve space, effective January 1, 1951.

The Journal Management Committee makes the following recommendations to the Executive Board:

1. That the charge of $5.00 per over-page on manuscripts exceeding 12 printed pages be continued.

2. That the Executive Board should make the decision regarding the establishment of sustaining memberships in the Association.

3. That the Secretary be authorized to change the paper stock used in the Journal.

4. That the Secretary be authorized to include order blanks for the ten-year index when notices of membership dues are mailed out next fall.

The Journal Management Committee reports that a program has been outlined for exploring certain possibilities for increasing the coverage of dairy science literature in the abstract section.

In behalf of the Association the Journal Management Committee wishes to express appreciation and commendation to:

1. H. Macy for his outstanding contribution to the Journal in the organization of the decennial index, which is nearing completion.

2. I. A. Gould and I. W. Rupel for their editorial service.

3. P. H. Tracy, who was appointed by the Executive Board of the Association as affiliate member of the Management Committee to aid in promoting the advertising program, for his success in obtaining additional advertising accounts.

4. P. R. Ellsworth, Secretary-Treasurer, for his devotion to and promotion of the Journal activities.

5. F. E. Nelson, Editor, and his staff for their continued excellent work.

Respectfully submitted, G. H. WISE, *Chairman;* J. K. LOOSLI; W. V. PRICE

Upon motion duly seconded, the report was accepted.

COMMITTEE ON STUDENT AFFILIATE ORGANIZATIONS REPORT

This committee, composed of Elmer Thomas, M. L. Dawdy and H. B. Henderson, prepared and mailed to each of the dairy departments a questionnaire for the purpose of obtaining suggestions as to ways by which the student affiliate program could be strengthened. Forty-three replies were returned out of fifty-one questionnaires mailed out. These replies represented forty-one states. The committee desires to express its appreciation to those who were willing to assist by returning the questionnaire.

It is to be expected that the questionnaire as formulated could not be considered perfect or could it include all items on which information was desired. Study of the replies shows that the questionaire was far from complete. For example, in only a few instances were student opinions expressed. More student opinions as well as opinions of the faculty certainly should have been secured.

It should be pointed out that there are certain definite weaknesses in the present student affiliate program which, if not soon corrected, may have rather far reaching effect upon the future of the ADSA. The necessity for increasing the dues to student affiliates this year has resulted in more than a fifty per cent drop in the number of student affiliates. The very small number of student affiliate transfers to regular membership also suggests that a stronger tie between the students and the ADSA is urgently needed.

Through the questionaire an attempt was made to secure the opinions of faculty members with reference to the advisability of recommending the formation of a national organization of dairy students. Such a plan has been suggested by many individuals and was considered by the committee to offer good possibilities as a means of strengthening the student affiliate program. It can be briefly stated that a wide variety of opinion was expressed on this point. About fifty per cent of the replies voiced approval of the suggestion, some with much enthusiasm. Some of those who expressed opposition to the suggestion gave as their reason that students are "organized to death", that such a move would mean "more work for staff members", and that a strong local organization is more to be desired than a national organization. Obviously, with this wide divergence of opinion, the committee is not in position to make a recommendation on this point at the present time.

The committee recognizes the fact that there would be many advantages to be gained by having a strong national organization of dairy students, so organized that it would have national student officers and an anuual meeting of delegates of the various clubs. Furthermore, the committee is quite aware of many of the problems which would be encountered in conducting such a program. No attempt will be made in this report to outline the advantages and disadvantages of this plan. It should be stated, however, that it is the belief of the committee that training students for leadership is certainly an important part of the job of dairy teachers and that no effort should be spared in accomplishing this objective.

In view of the fact that the committee is not satisfied with the results obtained this year, it is recommending to the Executive Board of the ADSA that the study of the Student Affiliate program be continued for at least 1 additional yr. It also is recommended that a meeting of faculty advisors of student clubs be held at the time of the ADSA meeting in June, 1952. At this time a very worthwhile discussion of the student affiliate program could be held under the direction of the Student Affiliate Committee.

In the meantime, however, it is the opinion of the committee that certain steps can be taken which will greatly improve the relationship of the ADSA with the dairy students. The following suggestions are offered:

(1) That the President of ADSA, at the beginning of the fall session of school, send a special message (even mimeographed) to each member of a local club through the faculty advisor explaining the program of the ADSA and its interest in college students.

(2) That in addition to receiving the Journal of Dairy Science, each student affiliate should be presented a membership certificate.

(3) That consideration be given to the possibility of the Secretary preparing a student news letter at least once each quarter, a copy of which would go to each member of the local club.

(4) That the Secretary of the ADSA be instructed to mail to the head of each dairy department at periodic intervals (possibly each quarter, or certainly during the fall quarter) forms for reporting the names of student affiliates as well as other dairy majors who have graduated during the quarter or year. The Secretary then could send each of them a note of congratulation and an invitation to join the Association as regular members. It also is recommended that this list of graduates be printed in the December issue of the Journal.

(5) On the basis of the opinions expressed in replies to the questionnaire, it is the opinion of the committee that to be eligible for membership as a student affiliate the person should be regularly enrolled for not less than half-time study and have a rank not higher than that of assistant professor.

Respectfully submitted, E. H. THOMAS; M. R. DAWDY; H. B. HENDERSON, *Chairman.*

Upon motion duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE REPORT

WHEREAS: The 46th Annual Meeting of The

American Dairy Science Association has been held on the beautiful campus of the University of Tennessee, and

WHEREAS: The host institution undertook the task and responsibility of arranging the details necessary for the successful conduct of the annual meeting under difficult and confining conditions in relation to other responsibilities of the institution and

WHEREAS: The arrangements that have been made by the local institution have been more than adequate in every detail, thus permitting this association to hold one of its most pleasant and successful meetings enjoyed by men, women and children alike, now

Therefore, be it RESOLVED: That The American Dairy Science Association extends its sincere appreciation for the courtesies and services provided for by the University of Tennessee, to Dr. C. E. Brehm, President, University of Tennessee, to Dr. J. H. McLeod, Dean of the College of Agriculture and to his staff, and especially to Professor C. E. Wylie, Head, Department of Dairy Husbandry, and the staffs of The Dairy Husbandry and other Departments who contributed to making possible this successful meeting.

WHEREAS: Many commercial and civic organizations have contributed greatly to the success and enjoyment of the 46th Annual Meeting,

Therefore, be it RESOLVED: That The American Dairy Science Association express to these organizations its sincere appreciation.

WHEREAS: The Borden Company Foundation has for the fifteenth year made available for presentation by The American Dairy Science Association the Borden Award for outstanding research in dairy production and dairy manufacturing,

Therefore, be it RESOLVED: That The American Dairy Science Association express its appreciation to The Borden Company Foundation for the Borden Awards in dairy production and dairy manufacturing by which The American Dairy Science Association may recognize achievement in the fields of dairy production and dairy manufacture.

WHEREAS: The American Feed Manufacturers Association has again made available to the Association the American Feed Manufacturers Award for outstanding research in dairy cattle nutrition,

Therefore, be it RESOLVED: That The American Dairy Science Association express its appreciation to The American Feed Manufacturers Association for this award by which recognition is given to outstanding research in dairy cattle nutrition.

WHEREAS: The De Laval Separator Company has made available an award for outstanding achievement in the field of dairy extension,

Therefore, be it RESOLVED: That The American Dairy Science Association express its sincere appreciation to The De Laval Separator Company for the award by which The American Dairy Science Association may recognize outstanding work in the field of dairy extension.

WHEREAS: It is recognized by The American Dairy Science Association that the economic advancement of the dairy industry is primarily dependent on the public acceptance of milk and dairy products as a valuable food and

WHEREAS: A high degree of public acceptance of milk and dairy products depends mainly on the availability of adequate amount of these products that are of high quality and on publicity to acquaint the public with the values of these products,

Therefore, be it RESOLVED: That The American Dairy Science Association commend and urge the expansion and intensification of the various programs in operation in the various states directed toward the improvement in the quality of milk and milk products and of the programs of those organizations which have for their purpose acquainting the consuming public of the nutritional value and economy of milk and milk products as food.

WHEREAS: The Purebred Dairy Cattle Association has invited representatives of the Production and Extension Sections to meet with that Association in coordinating constructive efforts concerning problems common to both,

Therefore, be it RESOLVED: That The American Dairy Science Association commend the Purebred Dairy Cattle Association for its cooperative action and express its gratitude for the opportunity of working together toward a deeper mutual understanding.

WHEREAS: Proved sires are such an important factor in building up high producing dairy herds and

WHEREAS: Such sires are still not available in sufficient numbers for the best interests of the dairy industry,

Therefore, be it RESOLVED: That The American Dairy Science Association commend the Bureau of Dairy Industry of the U. S. Department of Agriculture for its helpful service in recording daughter-dam comparisons, and urge that all agencies concerned with the proving of sires enlarge this program as rapidly as is possible.

WHEREAS: Antibiotics have proven beneficial in the treatment of mastitis and

WHEREAS: Research has shown that milk obtained from treated quarters of the cow's udder contains appreciable quantities of antibiotics

through the sixth milking (72 hr. following the final treatment) and

WHEREAS: It has been shown, for example, that even small quantities of penicillin will inhibit or retard lactic acid production in the progress of manufacturing cheese, buttermilk and other dairy products which are dependent upon growth of lactic acid-forming bacteria and

WHEREAS: Substantial economic losses may result from the production of inferior products or from the total loss of milk intended for processing and

WHEREAS: It appears that the best approach to the solution of this problem lies in informing producers regarding these facts,

Therefore, be it RESOLVED: That The American Dairy Science Association give full support to the solving of this problem by:

(1) Urging all dairy associations that have dealings with milk producers to foster a program of producer education in cooperation with manufacturers of antibiotics, designed to give producers an understanding of the correct use of antibiotics.

(2) Urging manufacturers of antibiotics to omit or alter statements in advertisements which may be construed to imply that milk from treated quarters can be used without a waiting period after treatment.

(3) Asking state and federal agencies to give serious consideration to the enactment of laws and/or regulations requiring containers of antibiotic preparations intended to be marketed direct to farmers for mastitis control to carry the following statement in a conspicuous place on the label of the product "Important! No milk obtained from a mastitis treated quarter within 72 hr. (six milkings) after the last treatment with this product is to be marketed."

WHEREAS: A healthy livestock agriculture is a permanent, soil-conserving agriculture, and a type of agriculture that provides our people with the highest possible quality of human diet, and

WHEREAS: Certain livestock diseases, parasites, and injuries increase the cost of production, reduce the volume of production, and increases the cost of foods of animal origin, and

WHEREAS: The livestock industry lacks reliable morbidity and mortality data on the incidence and location of these diseases and losses, and

WHEREAS: Such morbidity and mortality data are essential to the effective prosecution of the control and eradication of these costly health hazards,

Therefore, be it RESOLVED: That The American Dairy Science Association support the establishment of a Section on Vital Statistics in the Bureau of Animal Industry, United States Department of Agriculture, and that copies of this resolution be sent to the Secretary of Agriculture, the Director of the Agricultural Research Administration, and the Chief of the Bureau of Animal Industry, United States Department of Agriculture.

WHEREAS: The recognized economy of forage crops for livestock feed has stimulated increased acreages of improved pastures throughout the nation, expecially in the Southeast and

WHEREAS: The loss of animals by bloat has increased correspondingly and

WHEREAS: This loss of meat, milk, leather and wool reduces both farm income and the supply of these vitally needed products and

WHEREAS: The full value of improved grasslands, which is partially offset by the hazards of bloat, cannot be realized with the presence of this menace,

Therefore, be it RESOLVED: That The American Dairy Science Association urgently recommend to the Agricultural Research Administration of the United States Department of Agriculture and to the Directors of the Agricultural Experiment Stations of the States, that immediate steps be taken to accelerate and expand research leading to the solution of the bloat problem in livestock through a coordinated effort of the appropriate agencies in the Agricultural Research Administration and State Agricultural Experiment Stations.

Respectfully submitted, G. W. Vergeront, *Chairman;* T. R. Freeman; R. E. Hodgson; E. G. Hood.

Upon motion duly seconded, the report was accepted.

REGISTRATION COMMITTEE REPORT

Registration for the 46th Annual Meeting of the Association totaled 970, including 159 women and 110 children.

These people came from 43 states. There were nine foreign countries represented.

A detailed breakdown by state appears below.

State	Men	Women	Children	Total
Alabama	4	3	3	10
Arkansas	3	–	–	3
Arizona	–	–	–	–
California	6	2	2	10
Colorado	–	–	–	–
Connecticut	4	–	–	4
Delaware	2	2	1	5
Florida	6	2	4	12
Georgia	6	2	2	10
Idaho	1	–	–	1
Illinois	72	30	23	125
Indiana	18	3	1	22
Iowa	18	3	–	21
Kansas	8	1	–	9
Kentucky	26	3	12	41
Louisiana	6	4	5	15

Maine	1	1	3	5
Maryland	16	4	–	20
Massachusetts	11	2	2	15
Michigan	17	8	3	28
Minnesota	23	8	4	35
Mississippi	6	2	–	8
Missouri	6	2	–	8
Montana	–	–	–	–
Nebraska	8	4	4	16
Nevada	1	1	–	2
New Hampshire	6	–	–	6
New Jersey	6	2	1	9
New Mexico	–	–	–	–
New York	42	16	9	67
North Carolina	21	2	3	26
North Dakota	31	1	2	34
Ohio	38	16	9	63
Oklahoma	7	1	–	8
Oregon	2	–	–	2
Pennsylvania	36	9	8	53
Rhole Island	–	–	–	–
South Carolina	7	2	–	9
South Dakota	1	1	–	2
Tennessee	120	3	–	123
Texas	7	–	–	7
Utah	1	1	1	3
Vermont	1	–	–	1
Virginia	4	5	1	10
Washington	9	1	–	10
Washington, D. C.	13	4	2	19
West Virginia	10	1	–	11
Wisconsin	51	6	5	62
Wyoming	1	–	–	1
Totals	683	158	110	951
Canada	4	–	–	4
Foreign	14	1	–	15
Grand Totals	701	159	110	970

R. L. LUSH, *Chairman*

Upon motion duly seconded, the report was accepted.

MEETING OF THE EXECUTIVE BOARD

The Executive Board transacted the following business:

Approved the minutes of the 1950 Annual Meeting.

Approved the Editor's Report.

Approved the Secretary-Treasurer's Report.

Approved the Journal Management Committee Report.

Approved the Auditing Committee Report.

Approved a budget for 1952 amounting to $41,000.

Approved the Resolutions Committee Report.

Elected R. E. Hodgson as a member of the Journal Management Committee to serve for 3 yr. (1952–53–54).

Re-elected F. E. Nelson as Editor for the ensuing year (1952).

Re-elected P. R. Ellsworth as Secretary-Treasurer for the ensuing year (1952).

Approved the Honors Committee selection of L. A. Rogers as Honorary Member.

Renewed Student Affiliate Branch Certificates for the University of Minnesota, Alabama, Polytechnic Institute, Michigan State College and Montana State College.

Issued a new Student Affiliate Branch Certificate to University of Idaho.

Appointed W. E. Krauss, Associate Director of the Ohio Agricultural Experiment Station, Wooster, as American Dairy Science Association representative, Division of Biology and Agriculture, National Research Council. (1952–53–54).

Voted to recommend to the membership that the President, on request of an Annual Meeting host institution, appoint a committee of three to consider the recognition of any outstanding person who might be suggested by the host institution, for recognition at the Annual Banquet. This committee to function for 1 yr. only. Further, that the Association go on record as favoring such recognition of outstanding persons.

Appointed L. A. Moore as representative of the Association on the International Grassland Conference Committee.

Voted to recommend to the Association that the Borden Award Committees be composed of five members rather than three, to better facilitate the choosing of the recipients of the Borden Foundation Research Awards.

Approved the Student Affiliate Study Committee Report and voted to recommend that the Secretary carry out those immediate actions recommended in the report; further, that the committee be continued.

Voted to recommend to the Association that the immediate past recipient of the A.F.M.A. award be automatically appointed to the A.F.M.A. Award Committee.

The interim action of the Executive Board was approved on motion by W. E. Petersen and second by I. E. Parkin.

President Becker read a letter from The University of California inviting the Association to hold its 1952 Annual Meeting at that institution. The offer was accepted with thanks.

President Becker read a letter from The University of Wisconsin inviting the Association to hold its 1953 Annual Meeting at that institution. The offer was accepted with thanks.

Respectfully submitted,

P. R. ELLSWORTH, *Secretary-Treasurer.*

Upon motion duly seconded the report was accepted.

NECROLOGY COMMITTEE REPORT

Your Committee regrets to advise that during the past year the following members of the Association have passed on to their final resting place:

Arthur Devries Burke, technical editor for the Olsen Publishing Company for more than a quarter of a century, died of a heart attack at his home near Auburn, Alabama, August 16, 1950.

A native of Wheeling, West Virginia, he graduated from the University of Wisconsin in 1916; received his M.S. degree from Ohio State University in 1920; served as Assistant and Associate Professor at Oklahoma A. & M. from 1920–1929, and was head of the dairy department of Alabama Polytechnic Institute for 17 yr. thereafter.

At the time of his death, Professor Burke conducted the questions and answers department on technical problems for The Ice Cream Review and The Milk Dealer. He was author of books, "Practical Ice Cream Making" and "Practical Manufacture of Cultured Milks and Kindred Products."

He was President of the Southern Section A.D.S.A.; member Advisory Council, Sealtest, Inc., 1935–48; President Alabama Dairy Products Association; American Legion Commander; active member of the Kiwanis Club; member of Alpha Gamma Rho and Gamma Sigma Delta, and an Elder in the Presbyterian Church.

He is survived by his widow, Marjorie Burke, Annalue Farm, Auburn, Alabama.

Glenn G. Davis passed away January 26, 1950. He was born in Joplin, Missouri, on July 14, 1892. He was a student in the University of Missouri from 1911 to 1916. While a student, he started a purebred Holstein herd and Grade A dairy in Columbia, leaving school to devote part time to it. In 1927 he took over the management of the Fredmar Farm and purebred Holstein herd of Mr. F. W. A. Vesper, of St. Louis, until its dispersal in 1942. He became a member of the Holstein-Friesian Association of America in 1917, and served as a delegate from Missouri to the National meetings every year from 1922 to 1949. Mr. Davis served either as secretary or president of the Missouri Holstein-Friesian Association from 1921 to 1938, and managed the annual Missouri State Sale every year from its beginning until his death.

Mr. Davis was a member of Alpha Gamma Rho and the American Dairy Science Association and was a prominent figure in dairy organization work. Some of the positions he held were president of Missouri Dairy Association, vice-president and president of the Dairy Industry Council of Missouri, president of the St. Louis County Farm Bureau, manager of the Missouri American Dairy Association, and president of the Agricultural Alumni Association of the University of Missouri. His outstanding leadership and work with Holstein and general dairy organizations conveys some indication of his vitality, energy, unfailing courtesy and warmth of friendship which Mr. Davis radiated.

He married Tillie Grace Campbell on July 26, 1922. He is survived by her, two daughters and a son.

Kenneth Marvin Dunn, Assistant Professor, Research in Dairying at Michigan State College, was killed in an automobile accident on Sunday, July 30, 1950, near Millett, Michigan.

Mr. Dunn was born July 23, 1918, near Hastings, Michigan, the son of Mr. and Mrs. Harry A. Dunn. He was graduated from Michigan State College in 1942. During World War II, from 1942 through 1945, he served as a combat fighter pilot in the Pacific theater, attaining the rank of captain. He earned his Master's Degree from Michigan State in 1947.

Mr. Dunn joined the dairy staff at Michigan State January 1, 1946, upon his return from Army service. Mr. Dunn will be remembered for his research work on the nutritive value of corn silage and the role of soil fertility on the nutritive value of feeds for dairy cattle. He had completed most of the requirements for a doctorate degree under Dr. Huffman.

Mr. Dunn was a member of the Society of Animal Production, the American Dairy Science Association, Farm House Fraternity and Sigma Xi. He is survived by his wife Thelma, a 3-yr-old daughter Ann Marie, and a year-old son, Marvin.

Walter Lee Gaines (Dr.), Professor of Dairy Science, Emeritus, at the University of Illinois, Urbana, died November 20, 1950, in Urbana, Illinois. He was born in Crete, Illinois, on March 17, 1881, a son of Horatio N. and Martha Adams Gaines. He received his preparatory training in the local schools. He was graduated from the University of Illinois in June 1908, with the degree of Bachelor of Science in Agriculture, and was granted the Master of Science degree from that institution in 1910. From 1913 through June 1915 he was engaged in graduate studies in the fields of physiology and genetics at the University of Chicago, receiving the degree of Doctor of Philosophy in 1915. He joined the staff of the University of Illinois as an instructor in Dairy Husbandry immediately after he was graduated in 1908, and continued in the capacity until 1913. After receiving his Doctorate degree at Chicago in 1915, he returned to the home farm in Crete, Illinois, during the period before the United States entered World War I. However, in the summer of 1917, the Federal Government of the United

States appropriated emergency funds to the states for an increased food production program to be operated by the Extension Service of the United States Department of Agriculture. His home institution, the University of Illinois, called Doctor Gaines from his farm to aid in that program which gave encouragement to the development and expansion of the farm advisory system in Illinois. He served in the work of increasing food production until March 1918, when he returned to the Department of Dairy Husbandry as Associate Professor of Milk Production. In 1919 he was promoted to Professor of Milk Production which position he held until his retirement, September 1, 1949.

During the 30 yr. in which Doctor Gaines served as Professor at the University of Illinois, he contributed numerous fundamental ideas to dairying. He was the first to demonstrate the continuous formation of milk by the alveoli of the mammary gland and its accumulation in the udder. These facts were demonstrated by several means, including the intravenous injection of pituitary extracts, and became the basis for much of the later work ultimately resulting in present-day recommendations for the proper milking procedure with dairy cows. Primarily self-trained in mathematics, he used this science extensively in his researches involving the physiological function of milk secretion. Doctor Gaines was one of the foremost investigators in the field of milk secretion, and his contributions were highly regarded by scientists throughout the world. His "Fat-corrected milk" (F.C.M.) concept, for expressing in terms of milk containing 4 percent of butterfat the energy equivalent of the milk of any cow producing milk containing other than 4 percent butterfat, is almost universally used in the calculation of the results of milk production studies.

Doctor Gaines contributed extensively to the scientific literature of the relation between body size of cattle and their efficiency of milk production. He developed mathematically sound feeding standards based on feeding experiments of others and has shown that the currently acceptable method of standardizing milk production records of young cattle to an estimated production at mature age may not be biologically sound. His studies indicate that live weight changes during development constitute a far more important factor than the change in age alone.

Essentially a scientist, Doctor Gaines invented a number of pieces of equipment and calculators important in his studies. However, his interests went beyond the laboratory to the practical utilization by others of his scientific findings. Al though he was touchingly modest in his own appraisal of his contributions to science and the dairy industry, others recognized the highly meritorious nature of his work. In 1948, 1 yr. before his retirement from his University position, The American Dairy Science Association selected him as the recipient for the Bordon Award in Dairy Production, the highest honor given a scientist in his field, in recognition for his researches of the preceding 5-yr. period. The citation for this award aptly termed him "the modest leader in the United States of the scientific approach to the problems of milk secretion."

Doctor Gaines was the author of numerous articles published in scientific journals and in bulletins and circulars of the Illinois Agricultural Experiment Station. He was a fellow of the American Association for the Advancement of Science and a member of the American Society of Animal Production, American Dairy Science Association, the American Genetic Association, the Society of Sigma Xi, Alpha Zeta and Gamma Sigma Delta.

Linton Alexander Harris, Jr., was born in Fayette County, Georgia, August 27, 1924. Attended and graduated from Fayette County High School and attended the Martha Berry School at Rome, Georgia, for 2 yr.; transferred to the University of Georgia and was graduated with the B.S.A. degree in June, 1947.

He became Assistant County Agent of Wilkes County in March, 1948, and in September, 1949, became Farm Representative for the Washington Loan and Banking Co. in Washington, Georgia. He was an active member of the Methodist Church, Secretary of the local Masonic Lodge and also Secretary of the Lions Club in Washington, Georgia.

His death occurred in an automobile accident December 23, 1950, in Washington, Georgia. He is survived by his wife, the former Henrietta Davidson of Athens, Georgia, a 2-yr.-old son, L. A. Harris III; his parents, Mr. and Mrs. L. A. Harris, Sr., of Fairburn, Georgia; and one brother and one sister.

W. E. Sewell was an eminent teacher and research worker in the field of Animal Industry. At the time of his death in 1949 he was in charge of the animal research department of Proctor and Gamble Co., Ivorydale (Cincinnati), Ohio.

Dr. Sewell was born at Pratt City, Alabama, July 19, 1904. After completing high school, he entered the Alabama Polytechnic Institute, Auburn, Alabama, where he received the B.S. Degree in Agriculture in the Spring of 1924. From Auburn, Dr. Sewell continued his studies at the University of Missouri, where he received the M.A. Degree in 1925, and at Iowa State College,

where he was awarded the Ph.D. Degree in 1943.

Dr. Sewell joined the staff of the Animal Husbandry Department of the Alabama Polytechnic Institute, January 16, 1925, and remained with this institution continuously until December 31, 1945, except for a few months in 1939–40, when he was on leave doing special field work with the Farm Security Administration.

Dr. Sewell was known as a successful teacher and capable research worker. His research on the gossypol content of cotton seed meal attracted wide attention and led to his appointment as a member of the research staff of Proctor and Gamble Co. in 1946.

Dr. Sewell married Catherine McSween of Birmingham in 1926. In addition to his wife, Dr. Sewell left a daughter and a son.

George C. Supplee, former president of the International Association of Milk and Food Sanitarians (1925) died of a heart attack at his home in Bainbridge, New York, November 7, 1949.

Dr. Supplee was widely known for his chemical research work. He is credited with developing the first commercially practical method of irradiating milk.

He developed methods for the commercial isolation of riboflavin from waste milk products. For this work he received the Billings Award from the American Medical Association in 1936. In 1937, he received from the American Chemical Society the Borden Award for research in the chemistry of milk.

Dr. Supplee received his bachelor of science degree at Cornell University in 1913. He served on the Cornell faculty with special assignments for the United States Department of Agriculture in Pennsylvania, New York, California, Michigan and Minnesota. He received his Ph.D. degree from Cornell in 1919.

He was employed in 1918 to organize and manage a research laboratory for the Dry Milk Co., an affiliate of the Casein Co. of America. He continued as director of research until 1919 when this company was absorbed by the Borden Co. From 1929 to 1935 Dr. Supplee was directing head of numerous phases of technical and developmental research relating to milk products and derivatives under the Borden Co. From 1935 to 1944 he was director of the biological and chemical laboratories of the company.

In 1944 he organized the G. C. Supplee Research Corporation, with offices in Bainbridge, to render consulting services to the food and pharmaceutical industries. He was head of this corporation at the time of his death.

Philip Anson Wright, Chemist, Bureau of Dairy Industry, passed away in Washington, D. C., September 14, 1950. He was born in New Haven, Vermont, October 25, 1886.

Mr. Wright received his B.S. degree from Middlebury College, Vermont. He studied at Yale University during the next 2 yr. and, in 1911, was employed by the Dairy Division of the Bureau of Animal Industry. At that time he was transferred to the University of Missouri where he received the M.A. degree in 1913. He returned to Washington that year, where he resided until his death.

Much of his research concerned problems of butter and dried milk. He studied chemical aspects of problems relating to the deterioration of butteroil, butter and dried whole milk, and the variations in the chemical and physical properties of butter made from milk produced by cows on feeds differing in their type and percentage content of oils. He investigated the variations in the composition of milk, especially the variations in the butterfat of milk. His publication, "Testing Milk and Cream", has been distributed by the tens of thousands during the past 20 yr. He was author or co-author of 19 articles and publications.

Mr. Wright was a Mason, a Phi Beta Kappa and a member of The American Chemical Society, the American Dairy Science Association and the American Institute of Chemists. He was a member of the Calvary Baptist Church for more than 35 yr.

He is survived by his wife, Mrs. Isabel Wright, who lives at 215 Rock Creek Church Road, N.W., Washington, D. C., and a daughter, Mrs. Ellen Mary King, Portland, Oregon. A son Philip C. Wright, was killed in 1942 at Pasco, Washington, while serving as an instructor in a naval aviation unit.

Respectfully submitted,
W. H. Riddell
M. J. Reagan
J. M. Brown
G. H. Wilster, Chairman

Upon motion duly seconded, the report was accepted.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

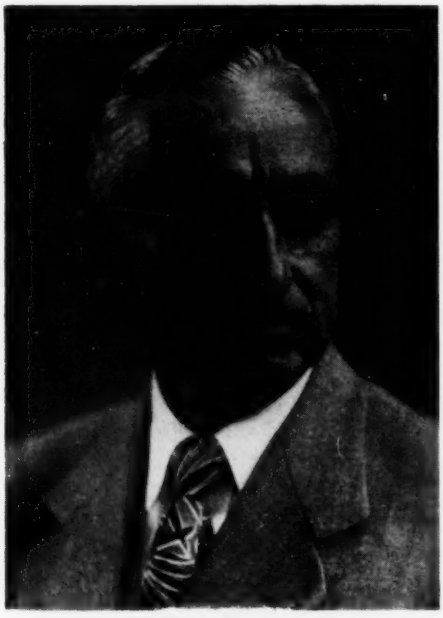
Knoxville, Tennessee, June 7, 1951

The Association Awards were presented in the Alumni Memorial Auditorium at 8:00 p.m. Toastmaster C. E. Wylie introduced President R. B. Becker, who presided at the Association Awards session. Awards were made as follows:

DE LAVAL DAIRY EXTENSION ACHIEVEMENT AWARD

W. E. Wintermyer, Chairman of the De Laval Dairy Extension Achievement Award Committee:

I was very much pleased when I received a letter from our President, Dr. R. B. Becker, advising that this year for the first time in the history of this organization an award will be given for outstanding achievement in dairy extension. I am sure this feeling is shared by



E. J. Perry

every member of the American Dairy Science Association and particularly the extension dairymen.

I also feel it a distinct honor to have had the privilege to serve as chairman of the committee and work with Prof. Fitch, Prof. Henderson, Prof. Gregory and Prof. Reeves in selecting the 1951 winner of this award. As you all know, the announcement of the establishment of the award was rather late and required some rapid decisions by the committee in order to get this information to the membership. In spite of this, eleven extension dairymen were nominated.

I am sure I speak for all extension dairymen when I say that it is very appropriate that a great organization like the De Laval Separator Company should sponsor the award as a living memorial in honor of Dr. Carl Gustaf Patrick De Laval, whose invention of the cream separator marked a historic milestone in the economic and social history of the world.

The De Laval Separator Company continued to carry on the great work of Dr. De Laval and after a number of years perfected the De Laval milker. We all appreciate the enormous contribution the milking machine has made in the saving of time and labor, in making dairying more attractive to farm youth, and in many other ways adding to the prosperity, well being, and efficiency of our great dairy industry.

In reading the history of the life and accomplishments of Dr. De Laval in the booklet "Ten Master Minds of Dairying" I was impressed by this paragraph. I quote, "Though he never became rich, he left the world richer becaues of his many contributions to practical mechanics. To prove that he was truly great we need only say that his entire life was a life of intelligent service to his fellowmen," end quote. Certainly this personifies an extension dairymen—I never have heard of one getting rich—but I know they all give unselfishly of their time to help their fellowmen.

The candidate for the first "De Laval Achievement Award in Dairy Extension" received his training at Pennsylvania State College and Columbia University. He has been a teacher, a county agent and dairy specialist for 35 yr. He has studied the dairy practices in Denmark and as a result of his studies and observations there he brought the idea of artificial insemination to this country and helped to establish the first cooperative artificial breeding association in America in 1938. This movement has spread to 47 states, Puerto Rico, Hawaii and Alaska. The artificial insemination program unquestionably is doing more to improve the breeding and production of the entire dairy cow population than any other dairy program in the history of our country. He is the author of two books and a number of bulletins and circulars.

The man that the committee selected to receive the award is Prof. Enos J. Perry of the New Jersey Agricultural Extension Service, New Brunswick, N. J.

Will Prof. Perry please come forward.

President Schmidt of the De Laval Separator Company, our committee presents as candidate for the "De Laval Achievement Award in Dairy Extension" Prof. Enos J. Perry.

Mr. Schmidt of the De Laval Separator Company presented Mr. Perry with a check for $1,000 and an inscribed, framed scroll.

AMERICAN FEED MANUFACTURERS AWARD

K. S. Morrow, Chairman of the American Feed Manufacturers Award Committee:

The 1951 award represents, for the fourth year, through the generosity of the American Feed Manufacturers' Association, recognition for outstanding achievement in research in Dairy Cattle Nutrition.

Publications during 1949 and 1950 resulted in nomination of seven candidates by the membership of our Association. Your committee augmented this list with three additional candidates. It was evident after a careful evaluation of the literature, that the choice must be made from

THOR W. GULLICKSON

a field of investigators whose contributions were of an exceptionally high order when judged on the four points established for consideration for this award, namely: originality, presentation of work, value to dairy cattle nutrition and benefits to and possibility of practical acceptance by the dairy industry.

The recipient of the American Feed Manufacturers' Association Award has long associated with investigations in the field of dairy cattle nutrition. Born in Wisconsin, he received his secondary school and college training in another great dairy state, Minnesota. After graduating from the University of Minnesota in 1918, he accepted a position as Assistant Specialist in Dairy Cattle Breeding with the Dairy Divison of the United States Department of Agriculture, Washington, D. C. In 1920 he returned to the University of Minnesota to accept a teaching and research assistantship and was granted his Master's Degree in 1922. A Ph.D. degree followed in 1934 from the same institution, where he has risen through successive ranks, to a professorship in the Division of Dairy Husbandry.

The research qualifying Dr. Thor W. Gullickson, the candidate for the 1951 American Feed Manufacturers' Association Award in Dairy Cattle Nutrition, was published in four journals during the prescribed period. In culminating 13 yr. of experimentation Dr. Gullickson and associates have demonstrated the lack of effect upon reproduction of feeding vitamin E-poor rations to dairy cattle during three generations. In addition, these researches have contributed much to the meagre information previously existing concerning the relationship of vitamin E nutrition to the utilization of carotene and vitamin A and to the general health, growth, and milk production of dairy cattle. The candidate made other contributions to our knowledge of calf feeding and to the testing of roughage quality in which an attempt was made to eliminate genetic influences by using identical twins.

Prior to the prescribed period the candidate made outstanding contributions to the knowledge of calcium and phosphorus nutrition, particularly as related to reproduction, to that of the nutrient requirements for growth and maintenance of dairy cattle and to that of calf feeding.

Will Dr. Gullickson please come forward? Mr. Diamond our committee presents as candidate of the American Dairy Science Association for the American Feed Manufacturers' Association Award—Dr. Thor W. Gullickson.

Mr. W. T. Diamond, American Feed Manufacturers' Association, presented T. W. Gullickson with a check for $1,000.

BORDEN AWARD FOR DAIRY MANUFACTURING

H. H. Sommer, Chairman of the Borden Award for Dairy Manufacturing Committee:

As chairman of the Borden Award Committee in Dairy Manufacturing it is my duty and personal pleasure to name the recipient of the award for 1951.

The man who has been selected for the award has been engaged in teaching and research in dairy manufacturing for 25 yr. In support of his nomination, more than one hundred publications, under his authorship or co-authorship, were cited. The scope of their subject matter indicates the broad interests of the recipient in dairy technology and dairy chemistry. In recent years much of his interest has been devoted to dry milk, and he has made important contributions to our knowledge of factors that affect the release of oxygen in the gas packing of dry milk, and factors that affect the keeping quality of this product.

The recipient was born in Weiser, Idaho, on September 15th, 1903. He received his B.S. degree from Oregon State College in 1925, his

M.S. degree from the University of Minnesota in 1930, and his Ph.D degree from the same institution in 1933. He has been at the University of Minnesota since 1925, where he has come up through the successive stages of academic rank to a full professorship in Dairy Husbandry in 1945.

S. T. COULTER

It is a pleasure to ask Dr. Samuel Todd Coulter to come forward to receive the 1951 Borden Award in Dairy Manufacturing.

Mr. W. A. Wentworth of The Borden Company Foundation presented S. T. Coulter with a check for $1,000 and a gold medal.

BORDEN AWARD FOR DAIRY PRODUCTION

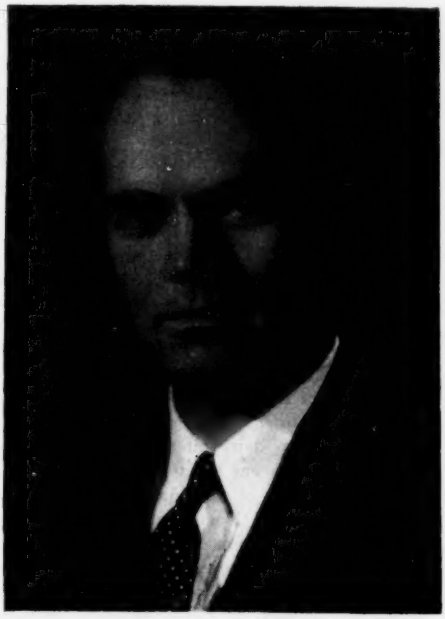
L. A. Moore, Chairman of the Borden Award for Dairy Production Committee:

The 1951 Borden Award recipient in Dairy Production of The American Dairy Science Association was selected for his fundamental research on the nutritional requirements and biochemistry of the minerals, fats, protein and vitamins with dairy cattle and other farm animals, more particularly for his studies on fat in the ration of dairy cattle, vitamin A in relation to calf nutrition; placental transfer and colostral storage of vitamins A and D; tocopheral relationships; rumen physiology and bacteriology, particularly in relation to cobalt, synthesis of amino acids and B_{12}, the role of inorganic sulphur; the influence of aureomycin on growth of calves and for his studies on the comparative nutrition of farm animals.

His name appears on some 89 scientific publications from 1934 to date. He is sole or senior author on 31 of these publications and 29 papers from 1945–50 deal directly with dairying.

In addition to the contributions made by these publications he also has made an outstanding contribution as a teacher and advisor of students. His lectures, where he expresses difficult facts and fundamentals in a manner that is easily understood but entirely complete, are a pleasure to attend.

He has shown excellent ability in guiding students in the proper paths without over-direction which has produced a desired balance of self-confidence and respect in the students. Graduate students in all fields of specialization have found guidance and obtained inspiration in their conversations and associations with him. He has always shown the true research spirit of cooperation, and his endeavors are given the highest respect by all his associates. He received the



J. K. LOOSLI

American Feed Manufacturers Award from the American Society of Animal Production in 1950.

The recipient was born at Clarkston, Utah, May 16, 1909, and received his B.S. Degree from Utah State Agricultural College in 1931, his M.S. from Colorado A. and M. College in 1932,

and his Ph.D. from Cornell University in 1938. He was an instructor at Utah State Agricultural College from 1933 to 1935; Assistant in Animal Nutrition at Cornell University from 1935 to 1938; with the Bureau of Biological Survey from 1938 to 1939; Assistant Professor of Animal Nutrition at Cornell University from 1939 to 1943; Associate Professor from 1943 to 1945 and was appointed Professor in 1945. At this time, in the absence of the Head of the Animal Husbandry Department at Cornell, he is Acting Head.

In behalf of this Committee on the Borden Award for Dairy Production and our associates in the Production Section of The American Dairy Science Association, it is a real pleasure to present Dr. John Kasper Loosli, Professor of Animal Nutrition at Cornell University to receive the award for 1951.

Mr. W. A. Wentworth of The Borden Company Foundation presented J. K. Loosli with a gold medal and a check for $1,000.

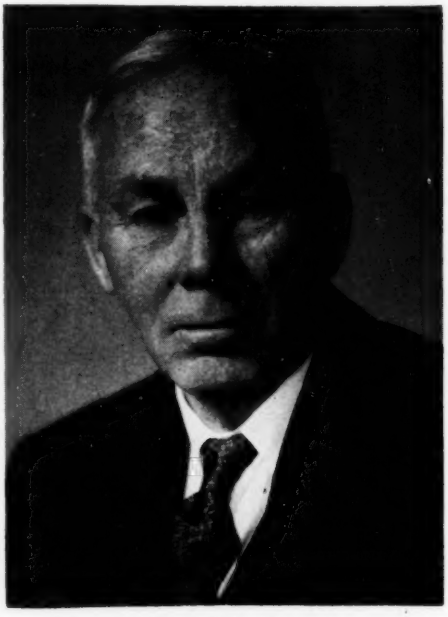
ASSOCIATION HONORARY MEMBER AWARD

P. H. Tracy, Chairman American Dairy Science Association Honor Award Committee:

Dr. Lore A. Rogers was born in Patten, Maine, February 7, 1875. He attended the University of Maine, obtaining the B.S. degree in Agriculture in 1896, and continued his education with graduate work in bacteriology and botany at the University of Wisconsin. He was an assistant in bacteriology at the New York State Experiment Station at Geneva from 1899 to 1902. From there he went to what was at that time the Dairy Division of he Bureau of Animal Industry of the United States Department of Agriculture. For the next 40 yr. he was actively engaged in research on dairy products and was Chief of the Research Laboratories of the Bureau of Dairy Industry during most of that period. In 1942 he retired from the Department of Agriculture and has since managed his own dairy interests in the town of his birth and adjacent territory.

The University of Maryland conferred the honorary degree of Doctor of Science on Dr. Rogers in 1923; the University of Maine also conferred on him the honorary degree of Doctor of Science in 1925 and, more recently, honored him further by naming its dairy industry building, Rogers Hall. He received the Borden Award in 1937.

Dr. Rogers has been one of the most active members of the Society of American Bacteriologists. He was its President in 1923 and was an associate editor of the Journal of Bacteriology. He is a member of The American Dairy Science Association, and has been an associate editor of the Journal of Dairy Science, a member of the American Public Health Association, of the Washington Academy of Science, the Washington Academy of Medicine, and of the Cosmos Club and an associate editor of Biological Abstracts. He was an official delegate to the meetings of the International Dairy Congress in Stockholm in 1911, in London in 1928, and Rome in 1934. For the World's Dairy Congress in Washington in 1923, he was chairman of the program committee, a job requiring arduous labor and heavy responsibility.



L. A. Rogers

Eighty-six papers bearing Dr. Rogers' name as author or co-author have been published in scientific journals or as bulletins of the U. S. Department of Agriculture. These papers deal with fundamental bacteriology and with bacteriology, chemistry and technology of dairy processes.

The principal contributions of Dr. Rogers to bacteriology are the taxonomy of various groups of organisms and studies of the factors controlling the rate of multiplication of bacteria and the limits of population in bacterial cultures. The latter work led to the development of a process for the manufacture of lactic acid by a continuous and rapid fermentation. His work on the causes of spoilage of butter led to the general practice of churning cream of low acidity and, later, to wide adoption of making butter from sweet cream.

He worked out a process for the preparation of a concentrated and self-preserving poultry

feed from sour skimmilk. This process has been successfully commercialized.

In his early work he was active in promoting the use of pure cultures in the manufacture of Swiss cheese and later in the manufacture of cheddar cheese from pasteurized milk. This latter work led to a process for curing and marketing cheddar cheese in consumer-size containers.

As evidence of the high esteem with which Dr. Rogers was held by his associates in Washington, the book "Fundamentals of Dairy Science" by the associates of Lore A. Rogers in the Research Laboratories of the Bureau of Dairy Industry, U. S. Department of Agriculture, was published in 1928. This book, one of the monograph series of the American Chemical Society, for years has been one of the leading reference works in the field of dairy science and is a fitting tribute to Dr. Rogers whose keen intellect and genuine enthusiasm for basic research led to his many scientific contributions to our industry.

Dr. Rogers was a pioneer in the study of applied problems in Dairy Bacteriology. This is best shown by his own quotation in the Biography of the late Chester Earl Gray. "For two years, we worked on the problem of the deterioration of butter in storage, making one lot experimental butter in the college creamery at Madison, but most of the time working in improvised laboratories in Wisconsin Creameries.

"Later we were back in Washington, where Gray set up his chemical laboratory in the kitchen of an old residence, while I grew my bacteria in the bathroom. In a woodshed remodeled into a dairy, we made butter on a very small scale."

Under such primitive conditions, L. A. Rogers started his highly successful career in the field of dairy research during which time he contributed much not only to our knowledge of dairy problems but to the building of young men who later were to become leaders in our industry.

It is very fitting, therefore, that The American Dairy Science Association whose purpose is to foster research and good teaching in the dairy field should pause to honor one of the greatest of its living pioneers, Lore A. Rogers.

Due to the inability of Dr. Rogers to be present, G. E. Holm accepted the Honorary Membership Certificate on behalf of Dr. Rogers.

INSTALLATION OF OFFICERS

President Becker installed the following officers-elect:

H. A. Bendixen (Washington), you are about to take over the responsibilities of President of The American Dairy Science Association. As President it will be your duty to be chairman of the Executive Board and submit to the Board for approval nominations of members to fill vacancies that may occur among the elected officers of the Association. As president you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now charge you with the honor of being President of The American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto.

H. B. Henderson (Georgia), you are about to take over the responsibilities of Vice-President of The American Dairy Science Association. As Vice-President, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Bendixen's term, you will automatically become President of this Association. I now charge you with these duties.

I. A. Gould (Ohio) and E. J. Perry (New Jersey), you were elected to the Board of Directors of The American Dairy Science Association. It will be your duty to pass on all applications for the establishment of divisions, sections and student branches of the Association. You will have full control of the budget and general business of the Association and have title to all property and funds of the Association. You will be members of a Board that has all the rights and power vested in the by-laws of the Association. With these privileges, responsibilities and obligations your are now considered as members of the Executive Board of The American Dairy Science Association to serve a term of three years.

RECOGNITION AWARD

President Becker called on H. B. Henderson who spoke as follows:

"It is a rare occasion indeed for a farm boy from the hills of East Tennessee to be asked to return to his alma mater to participate in the dedication of one of the most modern dairy buildings in the entire country. However, that was my privilege in November, 1949, on the occasion of the dedication of the new dairy buildings here on this campus. I hope all of you have visited these two modern structures during your stay here on the campus of the University of Tennessee. One of these buildings was named McCord Hall in honor of the person who, because of his untiring efforts in promoting the dairy industry, was able to bring this dream into reality.

McCord Hall will serve as a monument to the Honorable Jim McCord during the years ahead, but tonight it is our pleasure to have Mr. McCord

as an honor guest here at this 46th annual meeting of the American Dairy Science Association. Again, it is my rare privilege to participate in this program which refers to the extensive work of this great man who has meant so much to the dairy industry, not only of Tennessee, but of the entire nation.

To give a detailed statement of the accomplishments of Mr. McCord, or the contributions which he has made to the welfare of his fellow men, would consume far too much time on this program. Suffice it to say that he has spent much of his life in service to the dairy industry. As a newspaper publisher he has used his influence to develop a sound dairy program in his community. The success of this effort is reflected in the fact that his home county—Marshall County, Tennessee—has more Jersey cattle per square mile than any other county in America. Mr. McCord is listed among the great dairy cattle auctioneers of America, and as such, has been instrumental in developing a code of ethics which has meant untold millions of dollars to the purebred dairy cattle breeders of this country. His high standards in this field have developed confidence on the part of both buyers and sellers at dairy cattle auction sales. As a statesman, Mr. McCord has been instrumental in bringing the dairy industry to the forefront in a remarkable way. The dairy industry can well afford to pay tribute to such great men who use their influence to build and strengthen an industry which means so much to the health and economy of our nation while serving in public life. In all of the many public offices which he has held, including that of Mayor of his home town of Lewisburg, Tennessee, for more than 25 yr., a member of the County Court of Marshall County for an even longer period, representative to the 78th Congress of the United States from the Fifth Congressional District of Tennessee in 1942, and Governor of Tennessee from 1945 to 1949, he has never for one minute allowed his interest in the dairy industry diminish. It was during his term as Governor of Tennessee that funds were appropriated for the construction of the magnificent dairy buildings on this campus. As evidence of his direct interest in dairying, I should add that Mr. McCord has, on several occasons, served as president of the Tennessee Jersey Cattle Club, and has just arrived in Knoxville from the annual meeting of the American Jersey Cattle Club in Columbus, Ohio.

Governor McCord, as a further token of appreciation for your contribution to the dairy industry, it is my privilege to introduce you to the members of the American Dairy Science Association assembled here in their 46th annual meeting, and to present you with this picture of the dairy building which bears your name. The inscription on this picture reads as follows:

McCord Hall

"To the Honorable Jim McCord, honor guest of the American Dairy Science Association, in recognition of distinguished service to the dairy industry, June 7, 1951".

VOLUME XXXIV AUGUST, 1951 NUMBER 8

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

388. Incidence and distribution of Brucella abortus in slaughtered Bang's reactor cattle. N. B. McCULLOUGH, Natl. Inst. of Health, Pub. Health Service, and C. W. EISELE and A. F. BYRNE, Dept. of Medicine, Univ. of Chicago. Pub. Health Reports, **66,** 11: 341–345. Mar. 16, 1951.

Br. abortus was recovered in 42 of 100 Bang's reactor cattle sent to packing plants for slaughter. The tissue showing the highest incidence of recovery of the organism was the supramammary lymph nodes with 28 positive cultures found. The next highest incidence was in the uterus with 15 positive isolations. In 29 animals the organism was recovered from sites other than the supramammary lymph nodes or uterus. These findings emphasize the potential exposure to *Brucella* by persons engaged in handling carcasses of Bang's reactor cattle. D. D. Deane

389. The occurrence and significance of agglutinins in the genital organs of Brucella-infected cows. A. JEPSON and T. VINDEKILDE, Royal Vet. and Agr. College, Copenhagen, Denmark. Am. J. Vet. Research, **12,** 43: 97–99. Apr., 1951.

The possibility of demonstrating uterine infection with *Brucella* by an agglutination test of uterine fluid before the blood test is positive was first demonstrated on 39 slaughterhouse cows. Three cows with blood titers of 0, 40 and 640 were found to have uterine titers of 160, 640 and 160, respectively. *Brucella* were isolated from the uterine mucosa. Cows with high blood titer invariably had a high uterine titer, so the method is most helpful in checking cattle in a herd negative to blood test. Tests were made in 2 such herds in which abortions appeared. Uterine fluid was secured from a tampon placed in the vagina. Several cows were found with positive uterine titers and negative blood titers. Two of these later aborted and others still pregnant have developed positive blood titers. Following abortion the uterine titer was very much higher than the blood titer. E. W. Swanson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

390. An evaluation of errors in the composition control of butter. E. W. BIRD and V. H. NIELSEN, Iowa State Coll., Ames. Am. Dairy Prod. Mfg. Rev., **13,** 6: 30–35. June, 1951.

Composition control involves the proper percentages of butter components during the commercial life of the butter, rather than solely at the time of removal from the churn. The authors point out and explain the relative importance of errors that may be made during the manufacturing process. In a churning of 1,000 lb. of fat, an error of 0.1% in the initial moisture or salt test and an error of less than 2 lb. in the amount of salt and water added will cause an error of 0.1% in the fat of the butter. An error in cream weight of 60–80 lb. (depending on cream test) also will cause an error of 0.1% in the fat of the butter. Relatively large errors (0.6%) can be tolerated in the vat test of the cream without giving an error above 0.1% fat in the butter. The addition of water late in the final working process, high initial moistures and short final working times contribute to composition changes following manufacture. Procedures and precautions are given for the fat test of cream and for determination of moisture and salt in butter.
T. J. Claydon

391. Control of butter quality. A. H. WHITE. Can. Dairy Ice Cream J., **30,** 4: 29–31, 84, 90. Apr., 1951.

The author gives a review of the factors that influence the quality of creamery butter. Some of the essentials for quality control mentioned are high quality cream supplies, butter manufacturing report, neutralization, pasteurization, churning, equipment and sanitation, water supply, packaging and laboratory service. H. Pyenson

392. Research improves butter quality. E. G. HOOD. Can. Dairy Ice Cream J., **30,** 1: 54–60, 74. Jan., 1951.

A review of the inauguration of the Dairy Research Division in Canada is given plus data of the research conducted on butter by the division. Some of the major problems which have received attention in the first 27 yr. are creamery sanitation, the cause and control of butter defects, wrapping and packaging, surface deterioration in storage butter, workmanship, laboratory control and molds and yeasts. H. Pyenson

393. Report on the preparation of butter

samples. A. L. WEBER, Food and Drug Adm., FSA, New York, N. Y. J. Assoc. Off. Agr. Chemists, **34,** 2: 243–247. 1951.

Results of a collaborative study of 5 methods for the preparation of butter samples for analysis are given. Comments and criticisms of the methods given by collaborators are presented.

F. J. Babel

394. Pressure release device for cream cans. R. T. DUNHAM. U. S. Patent 2,555,477. 1 claim. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1:130. 1951.

A small tube is provided on the neck of a milk can to maintain the air above the product in the covered can at atmospheric pressure.

R. Whitaker

395. Butter-dispensing device. E. M. MOSIER. U. S. Patent 2,554,953. 8 claims. May 29, 1951. Official Gaz. U. S. Pat. Office, **646,** 5: 1605. 1951.

A device for cutting butter into small pats as required for serving is described. R. Whitaker

396. Butter cutter. H. E. YOUNG and G. V. FARNHAM. U. S. Patent 2,553,621. 1 claim. May 22, 1951. Official Gaz. U. S. Pat. Office, **646,** 4: 1148. 1951.

Pats are cut from a quarter-pound stick of butter as desired by this device. R. Whitaker

397. A study of Canadian margarine with particular reference to its vitamin A content. T. K. MURRAY, J. H. MAHON, A. WRIGHT, R. A. CHAPMAN and J. A. CAMPBELL, Food and Drug Div., Ottawa, Can. Can. J. Technol., **29,** 3: 147–151. Mar., 1951.

Samples of margarine from 15 manufacturers have been analyzed for vitamin A, moisture, fat, curd, salt, ash and antioxidants. Thirty samples yielded the following average values: 15.11% moisture, 80.70% fat, 1.42% curd, 2.96% ash and 2.91% salt. The average vitamin A content of 10 manufacturers was 4062 I.U./100 g., while that for 5 small producers was below labelled potency. Qualitative tests for 5 types of antioxidants indicated that none of these were present. Vitamin A was estimated by the antimony trichloride method. O. R. Irvine

CHEESE

A. C. DAHLBERG, SECTION EDITOR

398. Rind rot or wet end in Canadian cheddar cheese. E. G. HOOD, C. A. GIBSON and K. N. SMITH. Can. Dairy Ice Cream J., **29,** 12: 60–68, 78. Dec., 1950.

Rind rot is due to: (a) surface mold growth on the cheese due to lack of proper temperature and humidity control in the curing room sanitation; (b) improper rind formation; (c) wet headings and boxes; (d) paraffining and locking in excessive surface moisture which has accumulated from the preceding 3 causes or from condensation moisture due to temperature changes at time of paraffining the cheese. H. Pyenson

399. Bijdrage over de bereiding van smeltkaas (Contributions to the preparation of process cheese). H. HENDRICKX and A. DE VLEESCHAUER, Rykszuivelstation, Gent, Belgium. Proceedings of the 8th International Congress of Agricultural Industries, Brussels, July, 1950.

Cheese was processed at 75° C. for 5 min., using 25 g. citric acid and 0–25 g. Na_2CO_3/kg.; 300 ml. of water was added. Experiments were run with fresh and ripened Gouda cheese and with a mixture of 70% fresh and 30% ripened Gouda cheese. The best process cheese was obtained at pH values 5.5–5.6. At lower pH levels it was grainy with a strong acid flavor. At higher pH levels it was mealy with a bitter or soapy flavor. Fresh cheese melted sufficiently but lacked flavor; ripened cheese did not melt completely. The above mixture yielded process cheese of good quality. The processing temperature for the mixture was varied between 60–85° C. and the cheeses stored at 10, 20, 30 and 37° C. After 0, 10, 20 and 30 d. the quality was checked and the bacteria count determined. Storage temperatures of 30 and 37° C. were too high; at 10° C. the quality was still good after 30 d. Processing below 80° C. gave increasing bacteria counts with decreasing temperatures. These counts all showed considerable increases during storage.

A. F. Tamsma

400. The occurrence and survival of Brucella abortus in Italian cheese curd made from raw and pasteurized milk. H. L. GILMAN, N. Y. State Vet. Coll., Ithaca, and J. C. MARQUARDT, Dept. of Agr. and Markets, Albany, N. Y. J. Milk & Food Technol., **14:** 55–56 and 50. Mar.–Apr., 1951.

It has been reported that *Brucella* organisms survived the temperature used during the manufacture of Italian cheese from infected raw milk. After pasteurization of the milk, no viable *Brucella* organisms could be recovered from either the milk or the curd prepared from this milk. The authors strongly recommend that all milk should be pasteurized before manufacture into cheese as a safety precaution from the public health standpoint. H. H. Weiser

401. Cottage cheese provides further source of valuable milk bacteria. G. NEWELL. Food in Canada, 11, 3: 16, 18. Mar., 1951.

Yogurt culture replaces the regular starter in manufacturing cottage cheese at a Toronto company. The "short-time" method is used together with a coagulator, but specific details of the method are not given. O. R. Irvine

402. Report on gums in soft curd cheese. M. J. GNAGY, Food and Drug Adm., Los Angeles, Calif. J. Assoc. Off. Agr. Chemists, **34,** 2: 361–368. 1951.

A collaborative study was made of a revised method used to detect gum tragacanth, gum karaya and carob bean gum in creamed soft curd cheese. The revised method is outlined in detail. The method outlined had a sensitivity of approximately 0.05% for gum tragacanth and carob bean gum and a sensitivity of about 0.1% for gum karaya. F. J. Babel

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

403. Report on ash and acidity of milk and evaporated milk. G. G. FRARY, State Chemist, Vermillion, S. Dakota. J. Assoc. Off. Agr. Chemists, **34,** 2: 239–243. 1951.

A study was made of the acidity test for milk using the present method (Method A) recommended by A.O.A.C. (17.6 ml. milk diluted with an equal volume of CO_2-free water and titrated to the phenolphthalein end-point with 0.1 *N* NaOH; indicator used at the rate of 0.5 ml. of a 1% solution in neutral alcohol) and another method (Method B) using greater dilution and more indicator (17.6 ml. milk diluted with 2 volumes of CO_2-free water and titrated to the phenolphthalein end-point with 0.1 *N* NaOH; indicator used at the rate of 1.0 ml. of a 2% solution in neutral alcohol). Greater dilution of the milk sample and use of more indicator permitted earlier detection of the end-point and the pH of the neutralized solution was in the range of 8.0 to 8.3. It was recommended that the acidity test for milk be amended to conform to Method B.

Ash determinations made on samples of milk using 5- and 10-g. portions showed that equally satisfactory results could be obtained with the smaller sample. F. J. Babel

404. Process for stabilizing sterile evaporated milk. S. R. PARK (assignor to W. E. Mallory & Co.). U. S. Patent 2,553,783. 4 claims. May 22, 1951. Official Gaz. U. S. Pat. Office, **646,** 4: 1190. 1951.

Heat stability is imparted to evaporated milk by blending raw milk with a volume of specially treated milk, depending on the heat stability desired. The special treatment consists of heating some of the same raw milk in the range of 250 and 300° F. for about 2 sec. at the higher temperature or about 4 min. at the lower temperature to produce excessively stable milk. The mixture of milk is evaporated and sterilized in the conventional manner. R. Whitaker

405. Grade A milk powder. E. C. RITCHIE and G. SMITH, Danish Creamery Co., Fresno, Calif. Food Eng., **23,** 4: 110–111. Apr., 1951.

The requirements and process for the production of desirable nonfat milk solids are described briefly. The low temperatures used have no significant effect on the albumin and are mainly responsible for the desirable characteristics of the powder. The powder is particularly suitable for increasing solids-not-fat in fluid milk and in some kinds of cheese. T. J. Claydon

406. Process for the manufacture of a completely soluble highly nutritious product in powder form. F. NEEF. U. S. Patent 2,553,467. 13 claims. May 15, 1951. Official Gaz. U. S. Pat. Office, **646,** 3: 1010. 1951.

Coffee or tea is leached with citric acid, concentrated, neutralized and added to milk and sugar. The mixture is dried after being made slightly alkaline. R. Whitaker

407. Cottage cheese from skimmilk. E. B. COLLINS, U. of Cal., Davis. Milk Dealer, **40,** 7: 41–42, 124–126. Apr., 1951.

Satisfactory cottage cheese can be made from skimmilk supplemented with nonfat dry milk solids and condensed skimmilk. Data are presented showing the results of supplementing skimmilk with each of the above solids. With the addition of 6 and 9% solids as either condensed skimmilk or nonfat dry milk solids, the per cent of total solids recovered was greater than in case of either the use of no additional solids or the addition of 3% solids. However, the per cent recovered was no greater with 9 than with 6% added solids. This suggests that a maximum recovery is reached with the addition of about 6% solids. The per cent of total solids recovered was greater with condensed skimmilk than with nonfat dry milk solids, but the maximum recovery occurred upon the addition of about the same amount of solids with each concentrate. C. J. Babcock

408. Milk powder and its preparation. F. F. HANSEN. U. S. Patent 2,553,578. 5 claims. May 22, 1951. Official Gaz. U. S. Pat. Office, **646,** 4: 1137. 1951.

Milk powder is stabilized with calcium lactate and lacto-phosphate, corn starch and calcium sulphate in this method. R. Whitaker

409. Method of making lactose. A. E. WALLACE (assignor to M & R Dietetic Labs., Inc.). U. S. Patent 2,555,211. 10 claims. May 29, 1951. Official Gaz. U. S. Pat. Office, **646,** 5: 1672. 1951.

Sweet whey having a pH of no lower than 5.8 is treated with a cationic exchanger to replace Ca and Mg ions with alkalie metal or hydrogen ions, which reduces the pH to 4.0–4.7. The whey then is heated and the coagulated proteins are removed before concentrating and crystallizing the lactose. R. Whitaker

410. Method of making lactose. A. E. WALLACE (assignor to M & R Dietetic Labs., Inc.). U. S. Patent 2,555,212. 9 claims. May 29, 1951. Official Gaz. U. S. Pat. Office, **646,** 5: 1672. 1951.

Acid whey, having a pH of 4.0–4.7, is heated and the coagulated protein removed. The clear whey then is treated with an ion exchange material to replace the Ca and Mg with alkali metal or hydrogen ions. Lactose is crystallized from the condensed product. R. Whitaker

411. Method of making lactose. A. E. WALLACE and D. E. JAMES, JR. (assignors to M & R Dietetic Labs., Inc.). U. S. Patent 2,555,213. 14 claims. May 29, 1951. Official Gaz. U. S. Pat. Office, **646,** 5: 1673. 1951.

Whey is heated with lime at pH 5.8–6.8 to coagulate the protein. After filtering, the Ca and Mg are removed from the whey by a cation exchange material and the lactose is crystallized

from the concentrated product. R. Whitaker

412. Viscosity and density of aqueous lactic acid solutions. R. A. Troupe, W. L. Aspy and P. R. Schrodt, Univ. of Louisville, Louisville, Ky. Ind. Eng. Chem., **43:** 1143–1146. 1951.

This investigation was undertaken to provide accurate engineering data for the design of lactic acid plants and to determine the effect of a shift in equilibrium upon the viscosity of diluted lactic acid solutions. The viscosity values could be correlated in linear equations by plotting the logarithm of the viscosity against the logarithm of the absolute temperature. B. H. Webb

413. Whey product derived from milk and process of producing same. P. F. Sharp, D. D. Peebles, L. R. Hawk and A. M. Bisemer (assignors to Golden State Co.). U. S. Patent 2,555,514. 13 claims. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1: 139. 1951.

An edible product of pudding-like consistency is made by concentrating whey having a pH of 6.0–6.6 and having 1.5–3.0% of the solids as Ca to a total solids content of 25–55%. The heavy body is obtained by heating the concentrate to 200–280° F. R. Whitaker

414. Improved methods of recovering solids of cheese whey. E. D. McGlasson, Univ. of Idaho, Moscow. Am. Dairy Prod. Mfg. Rev., **13,** 6: 38–40. June, 1951.

The desirability of utilizing dairy by-products, the nutrient content of whey and the problem of sewage disposal make the utilization of whey as a food an important issue in the United States. Several methods of recovering whey protein were studied and the solubility of the final products compared. The methods involved the use of various ion exchange resins and precipitation agents, together with different drying processes. The passing of whey through ion exchange materials, precipitated by heat and $CaCl_2$, homogenizing and spray drying produced a product with the best solubility, although it was not comparable to non-fat milk solids. Preliminary tests on the use of the product in certain dairy and baking products gave encouraging results. Of the methods used for the recovery of lactose, it was found that whey treatment with ion exchange resins gave lactose of 93.9% purity, compared with 98.7% where no ion exchange materials were used. T. J. Claydon

415. Yoghurt cream. G. Jaggard and M. W. Ransom, Dairy Labs., Philadelphia, Pa. Milk Plant Monthly, **40,** 4: 54–56. Apr., 1951.

Eighteen per cent cream is pasteurized at 180° F. for 30 min., cooled to 140° F. and homogenized at 3,000 lb. pressure. Following cooling to 105° F., 1 qt. of Yoghurt starter is added to 40 qt. of the above cream and the product ripened at 95–100° F. for 6–8 hr. until an acidity of 0.65–0.70% is reached. The resulting product contains approximately 867 cal./pt. J. A. Meiser, Jr.

416. Het evenwicht der bacteriesoorten in yoghurt. (Factors influencing the proportion of streptococci and lactobacilli in a yoghurt culture.) (English summary) J. W. Pette and H. Lolkema, Nederlands Instituut voor Zuivelonderzoek, Hoorn, Holland. Neth. Milk & Dairy J., **5,** 1: 14–26. 1951.

The influence of incubation time, incubation temperature and per cent of inoculation was studied by varying 1 factor at a time, leaving the others constant. Increase in any 1 of the 3 factors mentioned favors the lactobacilli, which are more acid tolerant and have a higher optimum growth temperature than the streptococci. If these 3 factors are not balanced properly, then either cocci or rods will disappear after a period of time and in both cases the properties of the yoghurt will be affected adversely. The optimum proportion between rods and "diplococci" in a ripe yoghurt culture was found to be about 1:1.

A. F. Tamsma

417. Stevigheid en wei afscheiding van melk yoghurt. (Firmness and whey separation of milk yoghurt.) (English summary) J. W. Pette and H. Lolkema, Nederlands Instituut voor Zuivelonderzoek, Hoorn, Holland. Neth. Milk & Dairy J., **5,** 1: 27–45. 1951.

The firmness of whole milk yoghurt (2.5% fat) was determined and the influence of several factors investigated. As the temperature and acidity are important factors they were kept constant. Measurements were carried out at 18° C. and at an acidity equivalent to 95–100 ml. 0.1 *N* NaOH/100 g. of yoghurt. The milk was cultured at 45° C. Composition and treatment of the milk were found to be important. An increase in the total solids gave a firmer curd. Milk from the beginning of the lactation period caused low firmness, while milk from the end of the lactation period caused whey separation. Pasteurization showed different effects on different kinds of milk. For normal milk the best pasteurization seemed to be 5 min. at 85° C. Homogenizing generally had a beneficial effect on the firmness. It was found the best to do this after pasteurization and before inoculation. Small amounts of rennet worked favorably on firmness and whey separation; however, it adversely affected taste and texture. Addition of $CaCl_2$ or Na oxalate generally gave no improvement; however, in case of a Ca shortage as caused by excessive heating, addition of $CaCl_2$ worked favorably. No close correlation could be established between firmness and whey separation.

A. F. Tamsma

Also see abs. no. 436.

DAIRY BACTERIOLOGY

P. R. Elliker, Section Editor

418. Studies to formulate new media for the standard plate count of dairy products. L. Buchbinder, Y. Baris, E. Alff, E. Reynolds, E. Dillon, V. Pessin. L. Pincus and A. Strauss, N. Y. City Dept. of Health. Pub. Health Reports, **66,** 11: 327–340. Mar. 16, 1951.

Attempts were made to formulate a new medium for plating dairy products that would eliminate the difficulties encountered with cloudiness due to the added milk and the use of beef extract which is a non standard product of unknown composition in TGEM agar.

One successful medium contained Difco yeast extract, Difco proteose peptone, dextrose and agar. Peptones such as Difco tryptone, BBL trypticase and BBL polypeptone could be substituted for the proteose peptone. Other yeast extracts, however, could not be substituted satisfactorily for Difco yeast extract.

Lower counts were found when a BBL agar containing trypticase, phytone, dextrose, sodium citrate and sodium chloride was used in place of the standard TGEM medium. Lower counts also were obtained with a medium containing BBL yeast extract, BBL phytone, dextrose and sodium succinate. However, a BBL agar medium containing phytone, polypeptone and dextrose gave significantly greater counts than the standard medium. The addition of very small quantities of Na_2SO_3 and cystine to this latter BBL medium produced no further increase in counts. Substitution, omission or difference in amounts of certain ingredients were found to have predictable quantitative effects on counts.

The authors conclude that a medium to be satisfactory for use in the standard plate count must contain at least 3 different nutrients: a yeast extract (Difco) or a plant peptone (BBL phytone), an animal peptone and a carbohydrate. The authors presented a short method for testing the significance of mean differences of logarithms of paired plate counts. D. D. Deane

419. Agar plate method: progress report on media comparisons. Committee on Research and Standards, APHA. Am. J. Pub. Health Yearbook, **41**, 5: 72–74. May, 1951.

This report covers the work being carried out in an effort to find a plating medium to replace the standard TGEM medium for dairy products. On the basis of results obtained, the committee recommends the use of either Difco's tryptone glucose extract (milk) agar or BBL's trypticase glucose extract (milk) agar be authorized.

Investigations are in progress on 2 milk-free agars that might be suitable for plating milk. The average plate count with one of these was slightly below, and with the other slightly above, the desired range limit of $\pm 5\%$ that would assure little or no difference in numbers when compared to the count on TGEM. Further modifications of these 2 media are being studied in an effort to reduce this difference to a negligible amount. D. D. Deane

420. Standard plate count important in quality control. R. Smith. Can. Dairy Ice Cream J., **30**, 2: 64. Feb., 1951.

The standard plate count has emerged as one of the best methods for control of raw or pasteurized milk. This count and the coliform tests are the yardsticks of measuring existing conditions in a dairy. H. Pyenson

421. Capillary tube method for conducting the Brucella-stained antigen milk test. N. B. King, Ohio State Univ., Columbus. Am. J. Vet. Research, **12**, 43: 75–80. Apr., 1951.

The ring test has been modified to use capillary tubes rather than test tubes. Stained *Brucella abortus* antigen was prepared as for the tube method. Stained antigen was drawn 5–7 mm. into a 90-mm. capillary tube of 0.8 mm. bore, followed by milk to fill. The tube then was inverted and the lower end placed in plasticene allowing mixing of the antigen and milk. In milk with high agglutinin content a strong positive reaction is noted within 5 min. The method was compared with blood test and tube ring test on 428 cows from 9 herds. The ring tests and capillary tests were 96.3 and 93.8% efficient, respectively, in locating infected animals as shown by the blood test. The capillary test produced only 27 false positives compared to 69 for the ring test, showing that doubtful tests can be read more clearly in the capillary method. Other advantages of the capillary method are a saving of time (test may be read 1 hr. sooner), a saving in amount of antigen used, the possibility of using homogenized or skimmilk as well as whole milk, and eliminating cleaning expense by discarding the used capillary tubes. The capillary method is simple and practical and has a greater over-all agreement with the blood test than has the milk ring test. It is proposed that it could be used to detect many *Brucella* infections in individual animals. E. W. Swanson

422. The problem of antibiotics in milk. H. E. Calbert, Univ. of Wis., Madison. J. Milk & Food Technol., **14**: 61–64. Mar.–Apr., 1951.

Many kinds of antibiotics are being used at the present time in treating udder infections. Elimination of these products into the milk is recognized. Hence, the inhibitory effect upon starter culture organisms and consequent rendering them inactive is a serious problem.

The author suggests a comprehensive education program to inform the dairymen of the dangers involved in allowing these antibiotics to get into milk. H. H. Weiser

423. A report on the modified phosphatase method of detecting antibiotic substances in milk. E. S. Churchill, A. R. Drury, B. D. Lewis, C. L. Frank and C. S. Bryan, Mich. State Coll., E. Lansing. Milk Plant Monthly, **40**, 4: 28–31. Apr., 1951.

Experimentation on individual cows showed the presence of antibiotics in milk did not impair the accuracy of the regular phosphatase test. The Stoltz-Hankinson modified phosphatase test was not satisfactory in detecting presence of antibiotics in raw milk; rather, it indicated a variation in phosphatase content of milk from different cows. J. A. Meiser, Jr.

424. Destruction of lactic acid streptococcus bacteriophage by hypochlorite and quaternary ammonium compounds. R. B. Parker and P. R. Elliker, Oregon Agr. Expt. Sta., Corvallis.

J. Milk & Food Technol., **14:** 52–54. Mar.-Apr., 1951.

A comparison was made of the effect of sodium hypochlorite and quaternary ammonium compounds on the destruction of lactic acid streptococcus bacteriophage. Hypochlorite solutions in concentration of 50 and 100 ppm. destroyed the phage more effectively than quaternary ammonium compounds. However, at 200 ppm., both quaternaries and hypochlorites inactivated the phage in 15 sec. Phage strains for both *Streptococcus lactis* and *Streptococcus cremoris* showed different degrees of resistance to quaternary ammonium germicides. H. H. Weiser

425. A "loop" method for counting viable bacteria or bacteriophage. I. N. Ashaskov and F. C. Heagy, N. Y. Bot. Garden, N. Y. C., and Univ. of Western Ontario, London, Ont. Can. J. Med. Sci., **29,** 1: 1–4. Feb., 1951.

Two loops, the first of 0.3 mm. wire with an inside diam. of 1.5 mm. and the second of 0.5 mm. wire with an inside diam. of 3 mm., are used to transfer definite volumes of cultures to 2 dilution tubes and to spread fluid from the final dilution on an agar plate. Using 20 ml. of diluent and the 2 loops, counts with a ratio of 1 : 30 between successive dilutions may be obtained. The same technique is used for making clearing counts of bacteriophages. In this case, the final dilution from the bacteriophage suspension is made into a tube containing about 10^8 sensitive bacteria/ml. O. R. Irvine

426. Onderzoek over coliachtige bacterien in gepasteuriseerde melk (Investigation on coliform bacteria in pasteurized milk). (French summary.) A. Wilssens and A. de Vleeschauer, Laboratorium voor Landbouwtechnologie Rykslandbouwhogeschool, Gent, Belgium. Proceedings of the 8th International Congress of Agr. Inds., Brussels, July, 1950.

Three solid media, violet red agar, desoxycholate agar and brilliant green bile agar, were used for tracing coliform bacteria in pasteurized milk. The technic followed was according to the American "Standard methods for the examination of dairy products, 9th ed., 1948." Identification tests were based on the Imvic reactions. More than 90% of the typical looking colonies were coliform bacteria. The coliform flora of pasteurized milk was caused by contamination after pasteurization. *Escherichia coli* was found only in a few cases. Desoxycholate agar was found the best medium for tracing coliform bacteria in 1 ml. pasteurized milk. Based on selectivity, accuracy, easy technic and quick results, the solid media were preferred over the liquid media. A. F. Tamsma

427. Onderzoek over Escherichia coli in waters van zuivelbedryven (Investigation on Escherichia coli in water supplies of dairy plants). (English, French and German summaries.) A. Wilssens and A. de Vleeschauer, Laboratorium voor Landbouwtechnologie Rykslandbouwhogeschool, Gent, Belgium. Medelingen van de Landbouwhogeschool en de onderzoekings stations van de Staat te Gent, **15,** 2: 415–423. 1950.

In Belgium water supplies for dairy plants are investigated for *E. coli* by dividing 50 ml. of water into 5 tubes with brilliant green bile and observing gas production after 48 hr. at 37° C. For determination of *E. coli* the positive tests are inoculated in tryptone and the indol reaction employed. The water is considered unfit for use in dairy plants when gas is produced in more than 1 tube or when indol is formed from only 1 tube. The authors investigated the percentage of positive indol tests that were due to *E. coli.* Identification tests were based on the Imvic reactions. More than 100 water samples from about 70 dairy plants were investigated. Of the 84 positive indole tests, 45 were confirmed as being *E. coli.* About 30% of the waters with 1 positive tube of the 5 was judged incorrectly when only the indole test was used.

A. F. Tamsma

428. Cessation of bacterial motility as a rapid test for germicidal action. L. Buchbinder and P. Zaretshky, Bureau of Lab., N. Y. City Dept. of Health. Am. J. Pub. Health, **41:** 537–547. May, 1951.

A method is presented for the rapid determination of germicidal potency of fast-acting germicide solutions. The test consists of mixing equal volumes of a bacterial suspension of an actively motile coliform organism and the germicide solution to be tested. A hanging drop preparation is prepared immediately and examined with the aid of the microscope. Cessation of motility 30 sec. after mixing usually indicates a concentration of germicide that would kill most of the organisms in the same length of time. Results of this test correlate fairly well with those obtained with the percent kill test carried out in the laboratory. The test can be used in the laboratory and the field on chlorine compounds and all quaternary ammonium compounds.

D. D. Deane

429. The bacteriostatic effect of saturated fatty acids. J. B. Hassinen, G. T. Durbin and F. W. Bernhart, Wyeth, Inc., Mason, Mich. Arch. Biochem. Biophys., **31,** 2: 183–189. Apr., 1951.

Toxicity of the normal even-numbered saturated fatty acids from C_4 to C_{18} and oleic acid, toward different lactobacilli, *E. coli, A. aerogenes, S. faecalis,* and *P. vulgaris* was investigated in the search for factors contributing to the differences in the bacterial flora of breast-fed infants and that of infants fed cow's milk. C_4, C_6, C_{16} and C_{18} saturated fatty acids and oleic acid do not interfere with the growth of the bacteria tested. The bacteriostatic effect of C_8 through C_{14} acids for the gram-negative and gram-positive bacteria varied over a wide range, but the ratios of the number of moles of the fatty acids required for 50% inhibition in the case of each organism to the number of carbon atoms were similar. It was established that each increase in 2 carbon atoms increased the average molar toxicity 3.5 times;

however, molar solubility decreased on the average of 3.4 times with each increase of 2 carbon atoms over the range of C_8–C_{18}.

The more sensitive a bacterium is to the inhibitory effect of the C_{10} acid, the greater the number of fatty acids which will cause bacteriostasis. Gram-negative bacteria were least susceptible to the bacteriostatic effect of the C_8 and C_{10} saturated fatty acids and were not inhibited by the C_{12} or C_{14} members of the series.

Differences in the bacterial flora of breast-fed and artificially fed infants may depend upon the extent of fat digestion and absorption. It was suggested that dietary factors affording protection against saturated fatty acid toxicity may be important. H. J. Peppler

430. Comparison of citrovorum factor and a synthetic compound with Leuconostoc citrovorum growth activity. M. SILVERMAN and J. C. KERESZTESV, Natl. Inst. of Health, Bethesda, Md. J. Am. Chem. Soc., **73,** 4: 1897. Apr., 1951.

A concentrate of the citrovorum factor (CF), obtained from desiccated liver powder by fractional precipitation from Al_2O_3 columns was found by assay with *Leuconostoc citrovorum* 8081 to contain 176 CF units/γ, as compared with 152 units/γ of the synthetic compound resulting from the formylation and reduction of pteroylglutamic acid (PGA). Upon assay for folic acid activity using *Streptococcus faecalis* R, 1γ of the CF concentrate was equivalent to 0.648γ PGA, while 1γ of the synthetic product had a value of 0.572γ PGA. The purity of the CF concentrate was calculated to be approx. 70%. H. J. Peppler

431. Biological precursors of the pyrimidines. L. D. WRIGHT, C. S. MILLER, H. R. SKEGGS and J. W. HUFF, Sharp and Dohme, Inc., Glenolden, Pa., and L. L. WEED and D. W. WILSON, Univ. of Pennsylvania, Philadelphia. J. Am. Chem. Soc., **73,** 4: 1898–1899. Apr., 1951.

The role of orotic acid and ureidosuccinic acid, growth factors for *Lactobacillus bulgaricus* 09, in the biogenesis of the pyrimidine components of ribonucleic acid was determined by growing the lactobacillus in a pyrimidine-free basal medium containing orotic acid labeled in position 2 with C^{14} and DL-ureidosuccinic acid labeled in the ureido carbon atom. Counts of the purified purines and pyrimidine derivatives isolated from the harvested cells established that orotic acid serves as a source of the pyrimidine components of ribonucleic acid, and ureidosuccinic is an acyclic biological precursor of the pyrimidine ring. H. J. Peppler

Also see abs. no. 400, 401, 415, 416.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

432. Paper chromatography of casein and rennin. K. W. MCKERNS, Can. Packers, Ltd., Toronto. Can. J. Med. Sci., **29,** 2: 59–62. Apr., 1951.

Purified rennins of very high activity were prepared. The amino acid composition of the hydrolyzates of these preparations was studied by paper chromatography. Several fractions were obtained from the purified rennin by paper chromatography showing strong enzyme activity which was comparable to the density of their color development with a benzidine reagent. Whether these fractions represent distinct molecular species is not known as yet. The active enzyme was separated from the inert material of a crude commercial rennet powder by chromatography. The action of rennin on milk and casein also was studied by chromatographic techniques. It would appear that rennin causes an unfolding and breaking of the α-casein molecule with the appearance of several large polypeptides. No free amino acids were detected after rennin action on casein. O. R. Irvine

433. Report on detection of added water by the serum tests. H. J. HOFFMAN, Dept. of Agr., St. Paul, Minn. J. Assoc. Off. Agr. Chemists, **34,** 2: 248–251. 1951.

Analytical data obtained by use of the Cu serum, acetic serum and sour serum methods were used to construct a graph showing the reduction in immersion refractometer reading at 20° C. in relation to the % of added water in milk. Detection of watered milk using serum tests and A.O.A.C. minimum values is almost impossible except for the grosser adulterations. Any interpretation of serum refractometric data, with regard to added water in milk, was considered to be dependent upon the investigation of the serum of milk from the same source in its unaltered normal condition. F. J. Babel

434. Report on lactic acid in dairy products (chromatographic method). L. C. MITCHELL, Food and Drug Adm., Minneapolis, Minn. J. Assoc. Off. Agr. Chemists, **34,** 2: 352–360. 1951.

A method is outlined in detail for the determination of lactic acid in dairy products by a chromatographic method. The method as outlined was used in a collaborative study and comments of collaborators concerning the method are given. In general, the results obtained by the method were considered fair. Recovery of lactic acid from barium lactate averaged 88%. F. J. Babel

435. Chemicals introduced in foods. Committee on chemicals introduced in foods, APHA. Am. J. Pub. Health Yearbook, **41,** 5: 122–124. May, 1951.

A report is given of the progress being made in providing more adequate control over the use of chemicals in the food supply. D. D. Deane

Also see abs. no. 402, 403, 409, 410, 411, 412.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

436. Low-temperature concentration techniques in the dairy industry. D. ROSE. Can. Dairy Ice Cream J., **30,** 4: 50–52, 90. Apr., 1951.

The low-temperature evaporation technique, first applied to concentrated frozen orange juice

now is being used for the production of new milk products in the U. S. Many unanswered questions are being investigated in the National Research Council laboratories of Canada, in order to make it a more accepted product, although it has already gained a place in the retail market. Concentrated frozen milk also may play an important part in bringing about an increased consumption of dairy products, once research has developed a stable product economically. H. Pyenson

437. Concentrating apparatus. J. A. Cross (assignor to Mojonnier Bros. Co.). U. S. Patent 2,553,138. 7 claims. May 22, 1951. Official Gaz. U. S. Pat. Office, **646,** 4: 1285. 1951.

An evaporator in which the heating is accomplished by causing the product to pass between 2 heated corrugated plates, 1 of which is removable to facilitate cleaning, is described. R. Whitaker

438. Product control in Bowen-type spray dryer. Particle size and particle distribution. H. Wallman and H. A. Blyth, American Cyanamid Co., Stamford, Conn. Ind. Eng. Chem., **43**(6): 1480–1486. 1951.

One of the chief advantages of spray drying is the ability to obtain rapid, uniform drying of heat-sensitive materials. To better understand the spray drying process, quantitative data were obtained on the relative magnitude of the change in particle size that could be obtained with available operating variables of a Bowen-type spray dryer. A minimum deviation in particle size was desirable. Particle size affected the density, solubility, flowability and caking tendency of the product, sodium silicate. Particle size varied between 27 and 112 mμ and the standard deviation between 11 and 40 mμ. Variations in bulk density were recorded. B. H. Webb

439. Homogenization apparatus. J. Hault (assignor to B. Trudel and Cie). U. S. Patent 2,555,597. 4 claims. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1: 160. 1951.

Details are given covering a design for a head for a homogenizer suitable for processing dairy products. R. Whitaker

440. How to use cork for cold jobs. P. W. Swain, 330 W. 42nd St., New York, N. Y. Power, **95,** 5: 76–80. May, 1951.

Short descriptions and simple illustrations detail insulation applications. Among applications covered are cold pipes, pipe hangers, tanks, ducts, walls, columns, beams, concrete slabs, ceilings, roofs and floors. H. L. Mitten, Jr.

441. Ejector dumps ammonia. S. C. Page, Buffalo, N. Y. Power, **95,** 4: 121. Apr., 1951.

A water ejector was used to evacuate rapidly the ammonia and air from a large coil-type evaporator so that repairs could be made. After repair of the coil, the ejector also was used to remove air ahead of the ammonia for a quick return to service. H. L. Mitten, Jr.

442. Electric motor selection simplified. E. W. Fair, Bristow, Okla. Am. Dairy Prod. Mfg. Rev., **13,** 5: 18, 19. May, 1951.

During the emergency period, proper selection and care of motors is essential. Motors should be selected on the basis of the type of job involved. The different types of motors and their applications are described. T. J. Claydon

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

443. An investigation of the application of statistical quality control to dairy manufacturing. A. V. Moore and J. P. CoVan, Texas Eng. Sta., College Station. Mar., 1951.

The following types of data were obtained from commercial plants, covering periods of from 8–13 mo. and were treated statistically by the Shewhart control chart for variables: (a) bacterial plate counts of fluid milk, (b) butterfat losses in skimmilk made into cultured buttermilk, (c) cheese yield per 100 lb. of milk, (d) fat losses in buttermilk and (e) per cent fat in butter churned. The fraction defective chart method (or per cent defective chart) was used in checking for incidence of solder pellets in cans of evaporated milk.

The usefulness of the above 2 statistical quality control methods in the field of dairy manufacturers is discussed. The theory behind the methods used is explained. An example chart for collecting data is included. Formulas used in converting the data for presentation in graph form are shown. I. Peters

444. Automatic statistical control saves on package overfill. R. G. Merrill and W. Hecox, Batelle Memorial Inst., Columbus, O. Food Eng., **23,** 5: 55–59. May, 1951.

Since several million dollars worth of products are given away each year in overfilled packages, weight control is extremely important. Manual spot checking is not adequate for modern high-speed packaging lines. The Selectrol electronic check-weigher is an electronic computer and servo-control using statistical methods. It provides a high-speed control system that is economical and completely automatic. It will check up to 90 packages/min., and automatically adjusts the filler. Because of more accurate control and reduction in weight variations, the average package overfill can be reduced. T. J. Claydon

445. Most key markets now open to outside milk. Anonymous. Milk Dealer, **40,** 7: 40, 90–102. Apr., 1951.

A survey of milk ordinances indicates a trend toward removing restrictions against milk processed outside the city. Out of 40 representative markets contacted, 24 out of 29 replying reported their present ordinances do not restrict the market to local distributors, and that outside milk dealers may and do distribute milk in their markets. Among the few markets reporting ordinances which restrict milk distribution to milk pasteurized within the city limits or a short distance beyond them, one indicated outside milk is being distributed in the city despite the ordinance. An-

other reported the ordinance prohibited outside milk but was not enforced. Sixteen markets reported they accept on a reciprocal basis milk inspected by health departments of cities where the milk is pasteurized and packaged, although some indicated they also required certification by state health departments. Six revealed they required direct inspection of milk sources by the local inspection service; two reported they did direct inspection at present but were willing to work out reciprocal agreements with other markets; and one indicated milk will be accepted in the market during an emergency, even though it is inspected at the production source by outside agencies. C. J. Babcock

446. Efficient accounting system aids dairy operation. M. LECLERC. Can. Dairy Ice Cream J., **30,** 2: 40–72. Feb., 1951.

The installation of the I.A.I.C.M. system of financial and cost accounting has improved control of operations in all phases of the business in Canada. The system has improved general accounting and cost information. H. Pyenson

447. Economies in milk delivery. A. E. FRIEDGEN, 11 W. 42nd St., New York City. Milk Dealer, **40,** 8: 46–47, 62–66. May, 1951.

Data show the waste/route/year of 7 major items of delivery expense due to lack of proper cost control and maintenance. The 7 items are: gasoline, oil, sundries, tires, repairs, labor and body repair cost. The causes of waste and some of the preventive steps to avoid higher cost of operation are discussed. The difference between good and poor operation of delivery fleets of 50 routes amounts to $481.51/route/year or a total of $24,077 for the fleet per year. This is equivalent to the profit on 10.2 routes at 6% profit margin or on more than 20 routes at 2% profit margin. C. J. Babcock

448. Status of dairy research in Canada. D. B. GOODWILLIE. Can. Dairy Ice Cream J., **30,** 4: 36–37, 92. Apr., 1951.

There are many problems in the dairy industry in Canada and some require urgent and immediate attention. Government agencies must continue to play an important role in dairy research and the processing branch of the industry is interested in research. A committee on dairy processing has been established for the purpose of examining and reporting upon the status of dairy research in Canada. H. Pyenson

449. Effect of research on butter and cheese production. E. G. HOOD. Can. Dairy Ice Cream J., **30,** 4: 38–44, 92. Apr., 1951.

In dairy production research, greater stress is being placed on projects concerning breeding, feeding, management and sanitary milk production. In marketing and economics greater attention will be needed in the future to studies of potential markets, the possibility of increasing the demand for dairy products as a whole and the collection of statistical and economic information for the dairy industry at large. In the field of dairy processing or manufacture the Canadian dairy industry must meet the competition not only of dairy products from other countries but also of other foods. Investigations are under way for butter boxes and wrapping substitutes. H. Pyenson

450. Training men for the dairy industry. W. H. SPROULE. Can. Dairy Ice Cream J., **30,** 2: 27–28, 66. Feb., 1951.

Best education for work in the dairy processing industry is a combination of academic courses and practical plant experience. The training of dairy technologists—dairy plant managers and leaders, teachers and research workers—for the dairy industry is a cooperative effort between the college and the industry. The records show that many outstanding graduates have been lost to the dairy industry because of failure on the part of management to give proper recognition and to provide the opportunities of advancement. H. Pyenson

451. Role of the University in Canadian dairy research. D. L. GIBSON. Can. Dairy Ice Cream J., **30,** 4: 46–48, 60. Apr., 1951.

Universities have made important contributions to dairy research. At the University of Saskatchewan the research work being conducted for the past 4 yr. has been: (a) A method for utilizing whey from cheese as an animal feed supplement. (b) A study of body and texture of ice cream using combinations of sugars. (c) The efficiency of the new Shain Reagent for the determination of fat in dairy products. (d) Determination of pH in cheese. (e) Condensed frozen milk. (f) Solubility of dry milk solids. (g) Dehydration of butter. (h) Butterfat content of ice cream. (i) Chocolate dairy drinks. H. Pyenson

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

452. Stability of carotene and vitamin A in dry mixtures. M. E. WALL and E. G. KELLEY, Eastern Reg. Research Lab., Philadelphia 18, Pa. Ind. Eng. Chem., **43:** 1146–1150. 1951.

The stability of a number of vitamin A and carotene concentrates when mixed with dry carriers and when kept under varying storage conditions was determined. The reaction rate constants and the storage time for 50% loss in carotene were calculated and these provided a simple mathematical means for comparison of storage stabilities. When storage temperature was decreased, stability was increased. A concentration increase from 3,000 to 300,000 I. U. per pound greatly increased stability. The effect of the carrier on stability of the vitamin often was obscured by other variables. The effect of the vitamin source often was overshadowed by other environmental conditions. Relatively stable dry mixtures of high vitamin A potency can be prepared by using a proper combination of temperature, concentration, carrier and vitamin source. B. H. Webb

453. Spectrophotometric determination of beta-carotene stereoisomers in alfalfa. F. STITT, E.

M. Bickoff, G. F. Bailey, C. R. Thompson and S. Friedlander, Western Regional Research Lab., Albany, Calif. J. Assoc. Off. Agr. Chemists, **34,** 2: 460–471. 1951.

Beta-carotene in plant extracts consisted of a mixture of neo-B, all-trans, and neo-U stereoisomers. Separation of these stereoisomers and their determination was accomplished by a chromatographic procedure. A rapid spectrophotometric procedure agreed well with those of the chromatographic procedure. A method is given for the application of the spectrophotometric procedure to alfalfa meals. F. J. Babel

454. Report on vitamin A in mixed feeds. M. L. Cooley, General Mills, Inc., Minneapolis, Minn. J. Assoc. Off. Agr. Chemists, **34,** 2: 370–379. 1951.

A colorimetric procedure is outlined in detail for the assay of vitamin A. The method outlined is a modification and simplification of the previous method. Results obtained by 75% or more of the collaborators were as close to the calculated values as might be expected for this type of chemical assay. The method was considered valuable in estimating the vitamin A content of mixed feeds. F. J. Babel

455. Digestibility studies with ruminants. XV. The effect of the plane of nutrition on the digestibility of oats in an oats-hay ration. C. J. Watson, W. M. Davidson, J. W. Kennedy and P. E. Sylvestre, Science Service, Dept. of Agr., Ottawa, Can. Sci. Agr., **31,** 3: 113–119. Mar., 1951.

Digestibility trials were conducted on 6 grade Shorthorn steers using 6 rations of hay and hay with increasing quantities of Western Canada feed no. 1 oats. The coefficients of digestibility of the nutrients were calculated by difference from the mixed rations. As the quantity of oats increased their digestibility decreased, the decrease for total digestible nutrients being of the order of 5 absolute %, while that for protein was less and was not statistically significant. Similar trials with barley and linseed oil meal show that the effect of plane of nutrition is most marked in the case of barley and very slight with linseed oil meal. O. R. Irvine

456. The stability of various iodine compounds in salt blocks. W. M. Davidson, Muriel M. Finlayson and C. J. Watson, Science Service, Dept. of Agr., Ottawa, Can. Sci. Agr., **31,** 4: 148–152. Apr., 1951.

Iodine in 3 forms, potassium iodate, dithymol diiodide and an experimental iodide complex prepared by Merck and Co., was put up in 50-lb. salt blocks and subjected to outside exposure and manger feeding. The potassium iodate and dithymol diiodide blocks retained a high percentage of their original iodine content under both of these conditions. The iodide complex rapidly lost all iodine. Potassium iodate was physiologically available to swine and sheep. The methods of determining iodine in the above combination are described. O. R. Irvine

457. Biosynthesis of riboflavin, L. bulgaricus factor and other growth factors. Laboratory and pilot plant studies of biosynthesis by A. gossypii cultivated on grain stillage media. K. L. Smiley, M. Sobolov, F. L. Austin, R. A. Rasmussen, M. B. Smith, J. M. van Lanen, L. Stone and C. S. Boruff, Hiram Walker & Sons, Inc., Peoria, Ill. Ind. Eng. Chem. **43** (6): 1380–1384. 1951.

Grain alcohol stillage supplemented with glucose and animal stick liquor has been refermented with *A. gossypii* and this process was found to increase the riboflavin content of the stillage 1000-fold. Other nutritionally important factors, including vitamin B_{12} and an unidentified chick growth factor, were found. This factor may have a role in practical animal nutrition.

B. H. Webb

GENETICS AND BREEDING

N. L. van Demark, Section Editor

458. Some pathologic alterations of the bovine oviduct. L. Lombard, B. B. Morgan and S. H. McNutt, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **12,** 43: 69–74. Apr., 1951.

Gross and microscopic pathology of 154 repeat breeder cows with normal estrous cycles and apparent absence of genital abnormalities palpable per rectum (J. Dairy Sc., **32,** 237) was studied. Of 103 cows slaughtered 3 d. following insemination, 16 had oviduct pathologic alterations including 8 hydrosalpinx, 3 pyosalpinx and 5 chronic interstitial salpingitis. Of 51 cows slaughtered 20–35 d. following insemination, 6 had major pathologic changes including 2 pyosalpinx and 4 chronic interstitial salpingitis. Photomicrographs of typical pathological oviducts are presented. Ova were recovered from 2 of 3 cases of bilateral hydrosalpinx, from 2 of 5 cases of unilateral hydrosalpinx and from 2 cases of unilateral pyosalpinx in which the ovulatory side was not affected. Ova were not recovered in presence of bilateral pyosalpinx nor from 4 of 5 cases of chronic interstitial salpingitis. Some of the oviduct pathology also was connected with cases of purulent metritis or pyometra. Serosal nodules were present in 75 of the 154 cows.

E. W. Swanson

459. Bovine sterility and pregnancy in relation to the rheological properties of cervical secretions. F. A. Glover and G. W. Scott Blair. Nature, **167,** 4242: 285. 1951.

Preliminary results on 6 animals suggest the possibility of using the viscosity of the secretion taken from the mouth of the cervix as a means of testing for bovine pregnancy soon after conception and as a useful method for sterility or substerility prognosis. R. Whitaker

460. Computing inbreeding and relationship's coefficients from punched cards. L. N. Hazel and J. L. Lush, Iowa State College, Ames. J. Heredity, **41:** 301–306. Nov., 1950.

The authors present a method for adapting IBM and similar automatic machines for use in

computing coefficients of inbreeding and relationship. The adaptation to routine procedure saves labor because it is largely mechanical and clerical in nature. For this purpose Wright's formula is rewritten so that the decrease in heterozygosity due to inbreeding in a common ancestor is calculated indirectly rather than directly. Examples and references to the application of this adaptation are given. L. O. Gilmore

ICE CREAM

C. D. DAHLE, SECTION EDITOR

461. Use of corn syrup and corn syrup solids. L. G. DRUSENDAHL, Hubinger Co., Keokuk, Ia. Ice Cream Trade J., **47,** 3: 44, 45, 86, 88, 90–92. Mar., 1951.

The primary purpose of corn syrup solids in ice cream is to improve body and texture. Since corn syrup solids are neutral in flavor and low in sweetening value they may be used to build up mix solids. Regular corn syrup (C.S.U.), high conversion (acid) high conversion (enzyme) and dried corn syrup solids are available. To obtain a fine-textured, firm and chewy-bodied type of ice cream, about 5% corn syrup solids of the low conversion type should be used in combination with 12% sucrose. W. H. Martin

462. Push button handling of bulk dry sugar. Anonymous. Ice Cream Rev., **34,** 10: 45, 72, 74. May, 1951.

The Issaquah Creamery at Issaquah, Wash., is the first firm in the U.S.A. to employ an automatic system for handling bulk dry sugar. In this system, the bulk sugar is delivered to the plant in a special truck having a capacity of 20,000 lb. The sugar is discharged from the truck into a sugar storage bin (45,000-lb. capacity) located on the plant roof. The sugar as needed is discharged and automatically weighed into a hopper which can be swung over the mix vat by means of an overhead crane and dumped into the mix. This system, which was developed by the Amalgamated Sugar Co., eliminates the use of bags entirely, saves labor and storage space, protects the product against dampness and rodents and speeds up the mix making operation. W. J. Caulfield

463. Ice cream stabilizer. E. F. GLABE (assignor to Food Technol., Inc.). U. S. Patent 2,555,849. 11 claims. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1: 227. 1951.

An ice cream stabilizer consisting of approximately 7.65% carboxymethyl cellulose, 3.85% Irish moss, 0.38% soya whipping protein, 4.60% $KHCO_3$, 19% Na citrate and 64.52% partially refined corn starch containing some protein is described. The soluble Ca and Mg of the ice cream mix are pptd. by the bicarbonate and citrate. R. Whitaker

464. Extraction of alginates from Canadian seaweeds. R. C. ROSE, Natl. Research Labs., Ottawa, Can. Can. J. Technol., **29,** 1: 19–28. Jan., 1951.

Five abundant Canadian seaweeds were rated from a chemical standpoint as to their suitability as sources of alginate. Three species are apparently equal to seaweeds that are being used commercially elsewhere. Details are given of the methods of extraction, purification and yields. O. R. Irvine

465. Osmotic pressure, molecular weight and viscosity of sodium alginate. F. G. DONNAN and R. C. ROSE, Univ. of London, England and Natl. Research Labs., Ottawa, Can. Can. J. Research, **28,** 3: 105–113. Mar., 1950.

This work shows how the molecular weight of sodium alginate can be determined by measuring the values of the osmotic pressure, P, in the presence of low concentrations of NaCl, and extrapolation of P/C to $C = 0$, where C is the concentration of sodium alginate. For different samples of sodium alginate, the slope of the curve P/C against C was independent of the molecular weight. Molecular weight values from 48,000 to 186,000 were obtained. The intrinsic viscosity of the different samples was linearly related to the degree of polymerization. (Author's abs.) O. R. Irvine

466. Extraction, fractionation and evaluation of carrageenin. R. C. ROSE, Natl. Research Labs., Ottawa, Can. Can. J. Research, **28,** 6: 202–212. June, 1950.

The amount of carrageenin extracted from a sample of rinsed *Chondrus crispus* increased linearly with temp. of extraction up to 100° C., and more slowly thereafter up to 110° C. Heat treating carrageen or replacing the Ca and K in it with Na increased the yield of carrageenin at lower temperatures.

The viscosity of carrageenin extracts obtained at different temperatures increased and the concentration required to increase the viscosity of milk to 15 centistokes (*i.e.*, the "suspending conc." for cocoa in milk) decreased with temp. of extraction up to 60° C. Above this temp., the viscosity decreased and the suspending concentration increased. The increase in viscosity is attributed to the extraction of higher polymers of carrageenin with increased temp., the decrease to heat depolymerization during extraction.

Carrageenin, which was most effective in hot milk, had high suspending power compared with that which was most effective in cold milk. Concentrations required to give viscosities of 15 centistokes in milk were closely correlated to the same viscosity in 0.05 N NaOH solutions. O. R. Irvine

467. The determination of fat and sugar in chocolate. E. E. WOOD, Consumers Research Labs., Ltd., Toronto, Ontario. Can. J. Technol., **29,** 2: 66–70. Feb., 1951.

In determining fat, 5 g. of a properly prepared sample are extracted with 3 portions of petroleum ether, centrifuging after each extraction and decanting and combining the extracts. The solvent then is evaporated and the flask weighed to obtain the fat content.

Total sugar is determined on the residue after

the remaining ether has been removed. Fifteen g. of water are added to the dry residue and mixed; the suspension then is clarified by the addition of basic lead acetate. The total sugars are determined by refractometry measurements on a few drops of the clear liquid taken with a pipet from well below the surface. O. R. Irvine

468. "Certified food colors"—now government regulation. F. W. Atack. Can. Dairy Ice Cream J., **29,** 12: 76–78, 81. Dec., 1950.

Seventeen primary (or single) colors are now permitted for use as food colors in Canada, the same colors as in the United States. Before sale, all food colors that are synthetic dyes must be certified to the government of Canada as being of purity prescribed for use in and upon foods and must be sold in packages clearly labelled as food color. H. Pyenson

469. Better ice cream by fast, capacity-plus hardening. C. R. Havighorst, Food Eng. Staff. Food Eng., **23,** 5: 72–74. May, 1951.

Continuous hardening units increase freezing capacity by 275–300%. They contribute to superior quality and texture of the ice cream, due to much shorter hardening time and volume control during freezing. The equipment originally was developed for quick freezing fruits and vegetables and now has been modified for cartons. Present equipment is of 300-gal./hr. capacity. Rapid freezing is achieved by sliding cartons through channels of refrigerated metal. The unit is $7.5 \times 27 \times 9.5$ ft. and is installed in an insulated room with about 2 ft. access all around and on top. The 300-gal./hr. unit requires 8 tons of refrigeration operating at –30° F. Ammonia or Freon can be used. The equipment is made by Food Machinery and Chemical Corp., San Jose, Cal. T. J. Claydon

470. Code dating ice cream containers. W. H. Stevens, Supplee-Wills-Jones Milk Co., Philadelphia, Pa. Ice Cream Rev., **34,** 10: 46, 66, 68, 70. May, 1951.

Code dating of individual ice cream cartons affords the ice cream manufacturer a method for protecting quality of his product from the time it is produced until it reaches the consumer. The ice cream industry, until recently, has used code dating only as a guide to proper stock rotation in the hardening room. The code dating of individual packages can be of inestimable value in exercising proper control over stock turnover in dealers' cabinets. To be effective in this connection, it is essential that each individual carton and not merely the outer package be code dated. In this plant each carton is stamped with a number representing the day of the year when the ice cream was frozen.

Pint cartons are imprinted with the code date by means of a wheel-type printing device which is attached to the automatic filling equipment. Outer packages, gallons and half gallons can be coded by mounting the coder on the side or on top of the conveyor. Stamping bulk containers with the name and address of the manufacturer can be done with a special marking device mounted on the conveyor. This equipment reportedly saves expense over having the bulk containers printed at the factory. Details of the equipment are described. W. J. Caulfield

471. Attachment for mechanically agitating ice cream while being frozen in home freezers. F. A. Anderson. U. S. Patent 2,555,624. 3 claims. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1: 167. 1951.

A vertical-type small freezer is described, having a fan attached to the drive shaft for circulating the air surrounding the freezing chamber to facilitate rate of freezing. R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

472. 45% of wholesale fluid volume in United States is in paper containers. A. Steele. Can. Dairy Ice Cream J., **30,** 1: 70–72. Jan., 1951.

During the past 15 yr. the square paper bottle has been generally adopted by the industry. Paper packaging is highly sanitary. Paper containers are durable if properly packaged and conveyors are used in the plant. A much larger marketing area may be handled with the paper container, since there is less weight to haul from the plant and no return load. Approximately 29% of all milk consumed in the United States in 1950 was purchased in single service paper containers; approximately 28% of the fluid milk volume is wholesale and of this 55% is in glass and 45% in paper. H. Pyenson

473. 38mm bottle standard container in Britain. L. Chapple. Can. Dairy Ice Cream J., **30,** 1: 62–64. Jan., 1951.

Approximately 90% of the larger dairies of the United Kingdom have standardized on the 38mm bottle. The author believes it would be a grave mistake to switch from the 56mm to the 48mm bottle in Canada at this time since it appears the 38mm bottle is destined to be the universal standard for milk bottles in many countries. H. Pyenson

474. Checking up on quality. H. E. Calbert, Univ. of Wis., Madison. Milk Dealer, **40,** 8: 50, 112–116. May, 1951.

The results of a survey of the quality of market milk sold in retail stores throughout Wisconsin are reported. The quality of milk was determined by scoring it for flavor and odor, bacterial content, container and closure, temperature and sediment. All the samples were given full credit for temperature and for sediment. The most common flavor criticism was "oxidized", which was found in 34.4% of the samples. "Feed" flavor appeared in 31.6% and "cooked" flavor in 26.4% of the samples. Other flavor criticisms were "cowy", "scorched", "acid", "rancid", "lacked flavor" or "flat". 85% of the samples examined had a standard plate count of less than 30,000/ml. and the remaining 13% had standard plate counts of over 100,000/ml. Unprotected pouring lips were

found on 15.8% of the containers and 23.8% of the containers had partially protected pouring lips. The fat content did not enter into the scoring of samples, but it was found that the average test of all samples was 3.67%. The average for regular milk was 3.69% and the average for the homogenized milk, 3.62%. The results indicate that attention must be given to improvement in flavor of the finished product. Efforts must be put forth to overcome oxidized, feedy and cooked flavors and some attention must be directed to prevention of other off-flavors. C. J. Babcock

475. Refrigerated milk trucks. E. L. White, White Ice Cream and Milk Co., Wilmington, N. C. Milk Dealer, **40,** 7: 49, 58. Apr., 1951.

Experiments with 11 mechanical refrigerated delivery units showed that in addition to decreasing the cost of refrigeration, these units had these advantages: (a) By controlling the temperature within the body of the delivery unit, the milk was delivered to consumers at a temperature not exceeding 40° F. (b) The milk containers were free of dirt or other accumulated debris which frequently are present when wet ice is used. (c) Delivery units were cleaner looking. (d) The appearance of delivery salesmen was neater, as the trucks could be loaded when they came in from a day's run rather than in the a.m. just prior to going on the route. (e) The manual icing of the milk on trucks was eliminated. Following this experiment, all of the milk trucks were refrigerated, thus eliminating the ice bill which on the basis of the 1946 cost would have been tremendous. Furthermore, it eliminated the manufacture of ice in the plant, saving space for additional cooling rooms. All trucks are loaded at the end of the day's run with the next day's requirement and placed on the refrigeration line where thermo expansion valves regulate the temperature. A truck refrigerated in this manner for 8 hr. regardless of its size, retains an inside temperature of about 40° F. for 6–8 hr., which is more than enough to complete delivery at the last stop on any given route. C. J. Babcock

476. Milk cooler. R. W. Glaser, A. Olson and E. J. Olson. U. S. Patent 2,556,080. 4 claims. June 5, 1951. Official Gaz. U. S. Pat. Office, **647,** 1: 289. 1951.

To provide rapid cooling, aeration and deodorization of milk in a can, an air-driven motor is mounted in the lid which operates an impellor on a vertical shaft extending into the can. Exhaust air from the motor passes downward into the can to aerate the milk and to sweep out odors liberated from the milk. R. Whitaker

477. Tanker pick-up of milk from medium-size farms. A. C. Woodruff, Mojonnier Bros. Co., Chicago, Ill. Milk Dealer, **40,** 8: 48–49, 105, 106. May, 1951.

The bulk system for collecting milk from farms at the Green Valley Farms in Haddonfield, N. J., is discussed. The milk is picked up daily with a 2-compartment trailer tank, each compartment having a capacity of 1,500 gal. The number of cows milked per farm ranges from 14–48 with an average of 28, and the rated capacity of the producers' tanks in gallons ranges from 60–200, with an average of 142 gal. Some of the advantages of the bulk system are: (a) It has resulted in improving milk quality as evidenced by greatly reduced bacterial counts. (b) Milk comes into the plant at temperatures below 40° F. eliminating need for further refrigeration upon arrival. (c) Two men took 4 hr. (a total of 8 hr.) to pick up the milk at night in cans; for the equivalent amount of bulk milk, 1 man will take 6 hr. on the tanker, resulting in a labor time reduction of 25%. (d) The cost of operating can washing equipment and labor cost of personnel operating the can washing equipment are saved. (e) Valuable floor space has become available in the pasteurizing plant through release of can washing equipment. (f) The tanker is available for overnight storage of milk, as required on a 6-d. pasteurizing schedule.

C. J. Babcock

478. Onderzoek over het gebruik van Zuurstof by de pasteurisatie van de melk. (Study about the use of oxygen at the pasteurization of the milk.) (English, French and German summaries) A. de Vleeschauer, H. Hendrickx and M. Naudts, Rykszuivelstation, Gent, Belgium. Mededelingen van de Landbouwhogeschool en de onderzeokings stations van de Staat te Gent, **15,** 3: 1–54. 1950.

A study was made to determine if the use of O_2 according to the Wiser system, could increase the keeping quality of the milk, as it has with the Hofius-Richter process. (Experiments were carried out with 4 different technics.)

1. O_2 injection in the milk pipes between heater and regenerative section. The pasteurization apparatus could not resist O_2 pressures of 8 kg./cm.^{2} according to the Wiser system. Tests were run with Spirala, Plates and Stassano pasteurizers at pressures up to 4–5 kg./cm.^{2} and decreased milk capacity. Keeping quality tests were carried out in 3 different procedures. One lot was held at 50° C.; another at 15° C. and the third was held at 50° C. for 24 hr. and then held the remaining time at 15° C. The milk was examined for bacteria count, *coli* titer, acidity, boiling test, phosphatase and peroxidase tests and organoleptically. Results for treated and untreated milks were practically the same.

2. O_2 and milk were brought together at the bottom of an open cylinder placed between the preheating section and the clarifier. This cylinder was filled with glass beads and provided with an outlet through which the surplus O_2 escaped. Tests were carried out at 8 kg./cm.^{2} injection pressure and an O_2 consumption of about 1 l. for 1 l. of milk. No perceptible differences appeared in the bacteria count, *coli* titer and acidity. The organoleptic quality of the treated milk was slightly better.

3. Experiments were also carried out by using O_2 injection at 15 kg./cm.^{2} pressure and O_2

consumption of about 1 l. for 1 l. of milk in an open cylinder provided with partitions and placed between heater and regenerative section. Only 1 of the 10 trials gave a very good result; the others were a little better or gave the same result.

4. In order to obtain a better mixing of the milk with the O_2, technic 3 was modified by using a second cylinder with partitions. The outlet of the first cylinder was so arranged that all the time a gauge pressure of 1 kg. was used. Tests were run with injection pressures of 15, 12, 8 and 4 kg./cm.^{2} and the O_2 consumptions were held about 1, 0.75, 0.5 and 0.25 l., respectively, per l. of milk. At 15 kg./cm.^{2}, the treated milk kept better in storage at 15° C. Bacteria count and *coli* titer were lower most of the time and the milk sometimes kept 12–15 hr. longer. At 12 kg./cm.^{2} no difference was found. At 8 kg/cm.^{2} in general the treated milk kept longer at 15° C. At 4 kg./cm.^{2} no difference was observed except a slight improvement in the organoleptic quality.

Due to the irregular results of these experiments, the authors are still uncertain about the merits of the Wiser process. A. F. Tamsma

479. The creaming of milk. W. L. Dunkley and H. H. Sommer. Can. Dairy Ice Cream J., **30,** 4: 62, 70–74, 90. Apr., 1951.

The authors review the extensive literature (41 references) which has established the importance of the clustering of fat globules in the gravity creaming of milk. Creaming property determinations and clustering properties of the fat globules were measured on milk samples subjected to various treatments known to influence the creaming properties of milk. Pasteurization at temperatures higher than normal, dilution, homogenization and variations in the concentration of fat agglutinin influence the creaming properties of milk by modifying the extent of fat globule clustering. H. Pyenson

480. Methods for preparing cream for frozen storage. H. C. Olson. Can. Dairy Ice Cream J., **30,** 2: 58–62. Feb., 1951.

For best results, the following recommendations may be made in preparing cream for frozen storage: (a) Store only fresh cream, free from developed acidity. (b) Use every effort to eliminate metal contamination. (c) Pasteurize at a high temperature such as 170° F. for 20–30 min. or at 180° F. for 5 min. or longer. (d) Standardize the fat content to about 15% fat and then condense to about 40% fat and 20% serum solids. (e) Store in covered, well-tinned containers.

H. Pyenson

Also see abs. no. 433, 445, 447.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

481. Reactions of European and Indian cattle to wide temperature changes. S. Brody, A. C. Ragsdale, H. H. Kibler, C. R. Blincoe, H. J. Thompson and D. M. Sorstell. Fed. Proc., **10,** 1 (Part 1): 377. 1951.

The lag in critical temperature of Indian cows as compared with European cows appears to be associated with their greater heat dissipating surface area per unit weight and nature of skin and hair.

Various reactions when plotted against rising environmental temperatures show sudden changes occurring at 75° F. for European and about 70° F. for Indian cows. Only slight differences were observed on lowering temperature to 0° F.

S. Patton

482. Direct measurement of arterial and venous pressures in cattle using the electromanometer. A. F. Sellers and A. Hemingway, Minn. Agr. Expt. Sta., St. Paul. Am J. Vet. Research, **12,** 43: 90–96. Apr., 1951.

A method is presented for measuring the blood pressure of cows by means of an electronic apparatus connected to a direct-writing galvanometer. Use of plastic tubing from the vessel cannula to the apparatus gave suitable pressure tracings but failed to show a number of superimposed waves which were transmitted in lead tubing. Measurements were made on 105 dairy cattle showing diastolic pressures of 90–130 mm. Hg and 140–180 mm. Hg systolic pressure with 40–70 mm. Hg pulse pressure, all taken from the common carotid artery. Jugular venous pressures ranged from 2–10 mm. Hg. The taking of venous and arterial pressures also was followed by electrocardiograph recordings. The advantage of the method is the compactness of the apparatus and simplicity of operation, but a disadvantage is the long warm-up period required to secure stable readings. E. W. Swanson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

483. Reducing labor costs in cleaning of dairy equipment. W. E. Shiffermiller, Can. Dairy Ice Cream J., **30,** 3: 40–44, 78. Mar., 1951.

Hidden information difficult to bring out by any other method is obtained by use of time and motion study in analyzing cleaning operations. Good layout of equipment, sanitary lines and cleaning equipment aids in reducing cleaning time. The cleaning operation should be planned for each piece of equipment. Cleaning aids are sanitary pipe and fittings, racks for small parts, tables with wheels, wash tanks equipped with power driven solution-fed brushes and separator and clarifier disc washers. H. Pyenson

484. Sanitation control in ice cream manufacture. J. R. Jackson. Can. Dairy Ice Cream J., **30,** 1: 36–38, 72. Jan., 1951.

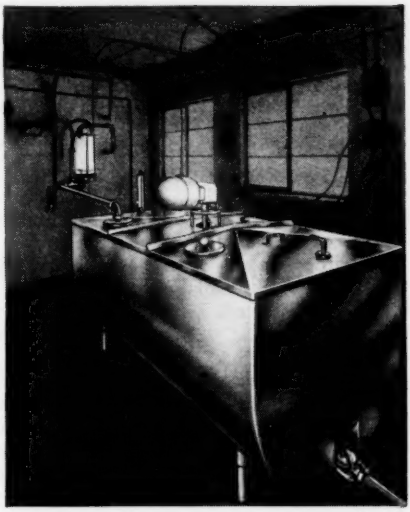
Sanitary control is more difficult in the ice cream than in the fluid milk industry. In sanitary regulations governing ice cream, one of the fundamental problems will be the establishing of tolerable bacterial plate count and *B. coli* minima. Points to consider in getting low *B. coli* counts are listed under equipment, supplies, personnel and general. H. Pyenson

THE BULK COOLING STORY

● Bulk cooling of milk on the farm is meeting growing acceptance in all parts of the country.

Farmers like it because it saves labor. More time can be spent in herd care. Bulk cooling also permits increases in herd size with the same labor. When tanker pick-up is used, stickage and drainage losses are saved for the farmer, and hauling costs are reduced.

Mojonnier Bulletin No. 240 contains photographs and diagrams of actual bulk cooling installations, plus descriptions of methods, and advantages to patrons and buyers.



Mojonnier 300 gallon Producers Cold Wall Tank on the Palmer Farm, Keota, Iowa.

WRITE FOR IT TODAY

Mojonnier Bros. Co. 4601 W. OHIO STREET CHICAGO 44, ILLINOIS

Marschall

RENNET and COLOR

Strong, clean Rennet is important in the making and curing of cheese. Use Marschall Rennet generously for maximum results.

MARSCHALL DAIRY LABORATORY, INC.
MADISON 3, WISCONSIN

FLAV-O-LAC FLAKES

UNIFORMITY IS IMPORTANT! Leading agricultural schools and colleges agree that the regular weekly use of FLAV-O-LAC FLAKES assures uniformity of smoothness, aroma and flavor in fermented milk products.

YOGHURT CULTURE

FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

SPECIAL FLAV-O-LAC FLAKES "40"

produce 40 quarts of starter on a single propagation. Single bottles $3.75. Plus postage. Free Cultural Manual of Fermented Milk Products on request.

THE DAIRY LABORATORIES DALARE ASSOCIATES

23rd & Locust Sts., Phila. 3, Pa.

BRANCHES

New York Baltimore Washington

See our catalog in Dairy Industries Catalog

Your advertisement is being read in every State and in 45 Foreign Countries

BACK COPIES of Journal May Be Available

The Association has available back copies of the Journal of Dairy Science. If you need back copies, please write and inquire as to whether the particular one that you need is available. In some cases we have only a few volumes and we do not sell them unless the complete set of volumes is purchased. In many cases we have six or eight volumes complete with 50 or 100 copies available of certain numbers such as the November or December issue.

Volumes	ADSA Members	Non-members and institutions
1–16 (if available)	5.00	6.50
17–32 (if available)	6.00	8.00
33–	8.00	10.00

If you are interested in procuring back copies please write to the Sec'y-Treas., American Dairy Science Assn., c/o Ohio State University, Columbus 10, Ohio. Make all checks payable to the

AMERICAN DAIRY SCIENCE ASSOCIATION

SUBSCRIPTION ORDER

To THE AMERICAN DAIRY SCIENCE ASSOCIATION
Publishers of the Journal of Dairy Science
Ohio State University, Columbus, Ohio

Please find enclosed Ten Dollars in payment of subscription to the *Journal of Dairy Science* for one year beginning with January, 19....

Name .....

Address .....

.....

Foreign postage 50 cents additional.

Checks, etc., should be drawn to the order of the *American Dairy Science Association* and forwarded to P. R. Ellsworth, Ohio State University, Columbus 10, Ohio.

It will PAY you to investigate—

the GBI VALUE LINE

of Ice Cream Stabilizers and Emulsifiers

In these days of high ingredient and operating costs, GBI stabilizers and emulsifiers demonstrate their value in short order. They earn their way and produce the type of ice cream, ice or other frozen confection you want. Whatever your product or operating conditions, there's a GBI stabilizer and emulsifier to give top performance and profit to you. The GBI VALUE LINE includes the following outstanding products—

Complete Stabilizer and Emulsifier—BODY-GUARD and PRIDEX
Straight Stabilizers—HY-GUARD and PRIDE
Straight Emulsifier—EMEX

WRITE NOW for complete bulletins and prices on the GBI VALUE LINE. These basic formulas cover the field. You will be surprised at their low cost.

Reg. U. S. GBI Pat. Off.

Manufactured by one of the largest established suppliers in the field of biological sciences.

GENERAL BIOCHEMICALS, INC. • 20 LABORATORY PARK CHAGRIN FALLS, OHIO

MINERALIGHT
Ultra-Violet Light
FOR DETECTING MILKSTONE, FATS AND OTHER SOILS

For Sanitarians, Field Men and Inspectors

Mineralight is a compact portable long wave ultra-violet light which causes fluorescence in milkstone, fats, and other soils not readily seen by the eye. Used like a flashlight. Operates 110 V-AC or batteries. Adapter available for 110 V-DC. Carrying case optional but necessary for battery operation. Moderate cost. Valuable aid to any size plant. Indispensable in improving sanitary standards. Write for literature.

KLENZADE PRODUCTS, INC.
BELOIT • WISCONSIN

"HANSEN'S"
DAIRY PREPARATIONS
For High Quality Dairy Products

CHEESE RENNET AND COLOR
COTTAGE CHEESE COAGULATOR
ANNATTO BUTTER COLOR
DANDELION BUTTER COLOR
CERTIFIED BUTTER COLOR
STARTER DISTILLATE
ICE CREAM COLOR
LACTIC FERMENT CULTURE

ODORLESS TYPE DAIRY FLY SPRAY
CULTURE CABINETS
TESTING SOLUTIONS

Write for Literature

CHR. HANSEN'S LABORATORY, INC.
MILWAUKEE 14, WISCONSIN

Your advertisement is being read in every State and in 45 Foreign Countries

The Secret of making
GOOD
CULTURED
DAIRY PRODUCTS
is yours with
FRIGIDRY
Reg. U.S. Pat. Off.

The original, the genuine
LYOPHILIZED DAIRY CULTURE
2 gm. bottle dry powder
makes 1 qt. Mother Culture

Manufactured Exclusively by
GENERAL BIOCHEMICALS, INC.
20 Laboratory Park . Chagrin Falls, Ohio

Copies of the 20-Year Index covering Volumes I to XX, inclusive are available at the following prices:

MEMBERS	
Cloth Bound	$2.35
Paper Bound	2.00

NON–MEMBERS	
Cloth Bound	$5.50
Paper Bound	5.00

Mention
JOURNAL OF
DAIRY
SCIENCE
When
Answering
Advertisements

This helps both the Advertiser and you

NOTICE TO CONTRIBUTORS

Authorship of Original Articles and Reviews.—Space in the JOURNAL is reserved for the publication of original research voluntarily submitted by members of the Association to the JOURNAL and review articles by invitation. In the case of joint authorship, the membership ruling applies to one author only.

Papers that already have appeared in print or that are intended for simultaneous publication elsewhere will not be accepted.

Manuscripts.—Manuscripts should be submitted in double spacing on one side of suitable $8\frac{1}{2}'' \times 11''$ paper. The original copy should be furnished. All illustrative and tabular material should accompany the manuscript.

Except in cases of invited reviews, papers must be limited to 12 printed pages unless previous permission from the editor is obtained. When non-review articles exceed 12 pages, a charge of $5 per over page is made.

Manuscripts will be published in the order of their receipt. They should be sent to the Editor, F. E. Nelson, Dept. of Dairy Industry, Iowa State College, Ames, Iowa. In order to speed publication, one author should be designated to assume the responsibility of checking the galley on all papers of multiple authorship. All galleys should be returned in the minimum possible time to avoid delay in publication.

Figures.—Original drawings, diagrams and charts should be done in India ink on tracing cloth (or white board) not larger than standard letter size ($8\frac{1}{2}'' \times 11''$). All lettering should be inked in block style and be of such size that the lettering will be not less than $\frac{1}{4}$ in. in height when the figure is reduced to 4 in. in maximum dimension. Typewritten labeling of axes and axis units is not acceptable. Original drawings should be submitted, rather than photographs of such drawings. When suitable drawings are not furnished, the author will be charged for the preparation of drawings of satisfactory quality by an independent agency.

Photographs.—Photographs for halftone reproduction should be glossy prints free of all imperfections.

Legends.—Legends for figures and photographs should be typed on a sheet separate from the illustrative material and should be made as concise as possible while retaining their descriptive character.

Tabular Material.—Tabular material should be clear, concise and accurate. Often data can be condensed and presented in summarized tabular form. Tables of only one or two lines should be avoided except in most unusual cases. Excessively large or complicated tables are almost impossible to print satisfactorily. Headings should be as concise as possible, yet descriptive in character. Data may be presented in either tabular form or in figures, but the same data must not be presented in both forms. Each table should be placed on a separate sheet and not in the body of the manuscript. The letters *a*, *b*, *c*, etc., should be used for footnote designations. If possible, tables should be so organized that they may be set across the page, rather than the length of the page.

References.—Literature reviews should be limited to only the most pertinent references. Reference lists should be double spaced and arranged alphabetically as to author and by chronological appearance of the journals cited under a given author. Papers by a single author always precede papers by that author and associates. References to multiple authors are arranged in the alphabetical order of the several authors. Give only initials rather than full first names of male authors. Citations in the text should be made by the number in parentheses, corresponding to the number in the reference list.

Each reference should contain the following: Reference number, author(s), title of article, name of journal, volume number, first and last page numbers, and year of publication. Titles of all articles should appear in complete untranslated form. Consult recent published articles in the JOURNAL for proper citation. Publications are abbreviated according to the form given in CHEMICAL ABSTRACTS, vol. 40, no. 24, part 2. 1946.

Sample of journal citation: (1) JONES, L. W., AND SMITH, J. D. Effect of Feed on Body of Butter. J. DAIRY SCI., 24: 550–560. 1941.

Sample of book citation: (1) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.

For Experiment Station publications, the citation should be as follows: (1) COULTER, S. T., AND JENNESS, R. Packing Dry Whole Milk in Inert Gas. Minn. Agr. Expt. Sta. Tech. Bull. 167. 1945.

The more common abbreviations used in the text are: cm., centimeter(s); cc., cubic centimeter(s); g., gram(s); mg., milligram(s); γ, microgram(s); ml., milliliter(s); mμ, millimicron(s); C., Centrigrade; F., Fahrenheit; lb., pound(s); oz., ounce(s).

Where configurational structures of chemical compounds are used, drawings suitable for reproduction by photoengraving are to be furnished by the author.

In preparing manuscripts, use of first person should be avoided.

DIFCO

CULTURE MEDIA
for Examination of Milk

BACTO-TRYPTONE GLUCOSE EXTRACT AGAR

is recommended for use in determining the total bacterial plate count of milk in accordance with the procedures of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association.

Upon plates of medium prepared from Bacto-Tryptone Glucose Extract Agar colonies of the bacteria occurring in milk are larger and more representative than those on media previously used for milk counts.

BACTO-PROTEOSE TRYPTONE AGAR

is recommended for use in determining the bacterial plate count of Certified Milk. The formula for this medium corresponds with that suggested in "Methods and Standards of Certified Milk" of the American Association of Medical Milk Commissions.

BACTO-VIOLET RED BILE AGAR

is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

BACTO-BRILLIANT GREEN BILE 2%

BACTO-FORMATE RICINOLEATE BROTH

are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

Your advertisement is being read in every State and in 45 Foreign Countries